EVIDENCE FOR GENETICALLY DISTINCT SYMPATRIC POPULATIONS OF ANADROMOUS AND NONANADROMOUS ATLANTIC SALMON, SALMO SALAR

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ABSTRACT

This study investigated the genetic relationship between sympatric anadromous and nonanadromous Atlantic salmon in the Gambo River system, eastern Newfoundland. Both forms were cultured in the laboratory and seasonal patterns of development were monitored and compared. Growth rates were not different during most of the first year of life, however smolting anadromous salmon (1+) grew more rapidly than nonanadromous salmon for several weeks prior to the time of seaward migration. Several other physiological parameters associated with the parr-smolt transformation were measured; no difference was noted in seasonal profiles of total body moisture, condition factor or plasma Na^+ and Cl^- concentrations. Both groups exhibited increasing levels of integumentary silvering during the late winter and early spring although this pattern was more marked among the anadromous group. Gill Na^+-K^+ ATPase activity increased over the same period in both groups, however mean activities among the nonanadromous salmon were consistently lower. Similarly, anadromous salmon displayed more, and larger chloride cells in the gill epithelium as revealed by light microscopy. Salinity tolerance was well developed in both forms in April and June despite differences in chloride cell abundance and Na^+-K^+ ATPase activity. Sexual maturation did not occur among female

postsmolts of either group nor among male anadromous postsmolts when cultured in freshwater and seawater. Most male nonanadromous salmon did mature as 'postsmolts'.

Mitochondrial DNA variation was also examined among wild salmon of both forms using restriction enzymes. The Atlantic salmon mitochondrial genome contains approximately 16,700 bp. No evidence was found for either length polymorphism or sequence heteroplasmy. Variable restriction fragment patterns were generated by five of eighteen enzymes; all variants could be accounted for by single base pair substitutions. Four distinct mitochondrial DNA genotypes were found. Pairwise sequence divergence estimates among genotypes range from 0.2 - 1.0 percent. Significant genotype frequency differences were observed among the two forms.

Results of this study are consistent with the hypothesis that anadromous and nonanadromous salmon in the Gambo River system represent genetically distinct reproductive units. Reproductive isolation is supported by the observation that the two forms use different spawning sites and that spawning times differ to some extent. Until further research is done to verify these observations it would be prudent to manage the two forms in this system (and possibly others as well) as separate stocks.

ACKNOWLEDGEMENTS

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This thesis is dedicated to the memory of David W. Anderson, a faithful friend, and Ralph M. Crockett, my grandfather.

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INTRODUCTION

For centuries the Atlantic salmon, Salmo salar L. has been an important component of the economies of countries bordering the North Atlantic Ocean. Historically the salmon occupied a much wider range than it does today. In North America this species could be observed in most rivers from the Hudson and others draining into Long Island Sound, north almost continuously to the watersheds flowing into Ungava Bay. European salmon occurred in rivers from the Iberian Peninsula north to the Pechora River, as well as rivers in the British Isles, Iceland and Greenland (Netboy 1968). The range has contracted considerably in recent times. Most New England rivers now support no salmon or only tiny remnants of former populations. Similarly, salmon have been extirpated from many rivers in the Maritime Provinces of Canada. Populations in Newfoundland and Labrador are smaller than in the past although most rivers still contain some salmon. The situation in Europe is more serious: salmon no longer occur in Switzerland, Denmark or the Low Countries. Remnant populations in Spain and France are seriously threatened. The Rhine, at one time Europe's most productive river, no longer supports salmon: only a century ago this river supported an annual catch in excess of a million pounds (Netboy 1968).

Several factors are responsible for the decline in Atlantic salmon populations. Damming of rivers for hydroelectric development has rendered large sections of many

formerly productive rivers inaccessible to upstream migrating fish. Pollution is a serious problem in industrialized areas, and overfishing has also contributed to the decline.

The life cycle of the Atlantic salmon is well known (Jones 1959). During the autumn mature fish bury their large yolky eggs in depressions excavated from gravel bars in freshwater streams. Embryonic development ensues during the winter and fry emerge in the spring to begin feeding. The young salmon remain in freshwater for a variable length of time, depending on temperature and food availability. This period may last for as little as one year in productive southern streams, or as long as seven or eight years in cold northern rivers. Juvenile salmon (parr) then undergo a metamorphosis that prepares them for life in the marine environment. This metamorphosis includes a series of physiological and behavioral changes collectively referred to as the parr-smolt transformation (reviewed by Hoar 1976, 1988; Folmar and Dickhoff 1980). Juvenile salmon that have undergone these changes are known as smolts, and at this stage movement downstream to the ocean takes place. Little is known about the postsmolt stage after entering the marine environment. Salmon that remain at sea for only one winter before maturing (grilse) probably do not move great distances from the natal river, while fish remaining for two or more winters at sea (multi sea-winter salmon) may travel great distances (Thorpe 1988). Many salmon from North America and

Europe migrate to feeding grounds in waters off west Greenland and Labrador. Other stocks, for example those in the Baltic and Bay of Fundy, do not undertake such extensive oceanic migrations (Huntsman 1939; Jessop 1976; Thorpe 1988).

Growth in the marine environment is rapid. Postsmolts captured along the north shore of the Gulf of St. Lawrence were calculated to grow at a rate of 1.65 mm/day during the first two months after leaving the river (Dutil and Coutu 1988). After only one winter spent feeding at sea, smolts originally measuring approximately 15 cm can typically attain lengths of more than 50 cm and weights of 1.5-3.0 kg (Allen et al. 1972). The adult salmon ultimately return to freshwater where they spawn, usually in their river of origin.

The life history described above represents that of the Atlantic salmon in a very general sense. Salmonid fishes as a group are well known for plasticity in life history parameters, and intraspecific variation is well documented. In addition to environmentally caused variation, important genetically determined variation in life history traits exists. For example, Naevdal et al. (1979) reported differences in postsmolt growth rates and age at first maturation among sibling population groups of cultured Atlantic salmon. Similarly, Sutterlin and MacLean (1984) showed that, despite similar growth rates, female Atlantic salmon from a dwarf nonanadromous population matured at age 2+; no females derived from anadromous parents matured at

this age. A significant proportion of male Atlantic salmon (and other species) mature as parr and spawn before smolting and migrating to sea (Jones 1959). This phenomenon is very widespread although the frequency of such 'precocious' maturation varies among populations (Dalley et al. 1983). Saunders and Schom (1985) described extensive variation in life history parameters of Atlantic salmon and suggested that intrapopulation heterogeneity in age at maturation prevents inbreeding depression in apparently small spawning populations. Since spawners represent different year classes, individuals can mate with members of cohorts other than their own, resulting in increased effective population size.

In contrast to salmon populations in which the major growth phase occurs at sea, many Atlantic salmon never leave freshwater. These nonanadromous or freshwater resident forms are common in many parts of the species' range and are known by several local names. The famous Sebago salmon are named for the lakes they occupy in Maine. Nonanadromous salmon in Newfoundland and parts of Québec are known as ouananiche. The latter are found in more northerly areas than Sebago salmon and do not usually attain such large size as the latter. In Norway, nonanadromous salmon are known as blege or smablank.

While nonanadromous forms are often referred to as landlocked salmon, many populations are not physically denied access to the sea, yet remain in freshwater throughout their life cycle. Landlocked salmon that are physically prevented

from entering the sea also exist. Power (1958) has suggested that the latter are of recent origin, and have evolved since the Wisconsin Glacial Period. Withdrawal of the continental ice sheets and the resulting upward rebounding of the earth's crust created barriers to upstream migrating anadromous salmon in many rivers. Power suggested that cool temperatures prevailing at this time resulted in slow growth and delayed smolting in salmon inhabiting these systems. Under these conditions maturation without prior smolting may have occurred in some females, resulting in evolution of the nonanadromous populations that now occupy sections of watersheds upstream of barriers to migration. Such populations are effectively isolated from genetic input from salmon downstream of the barrier.

The taxonomic status of nonanadromous salmon has been problematic; early authors granted subspecific status to these forms i.e. Salmo salar sebago and S. s. ouananiche (Jordan and Evermann 1896). Wilder (1947), however, was unable to find consistent morphological or meristic differences among the forms, and concluded that subspecific status is unwarranted.

The degree of anadromy among salmon in Newfoundland is variable. In addition to typical anadromous forms, physically landlocked salmon as well as nonanadromous salmon that are not physically landlocked are common. Several populations of physically landlocked salmon have been described, some of which contain only stunted fish. Sutterlin and MacLean (1984)

described one such population from Five Mile Pond East (Avalon Peninsula) in which fish seldom exceed 15 cm in length and females spawn for the first time at age 3+. Leggett and Power (1969) described a similar population in Flatwater Pond and Barbour et al. (1979) described another from Candlestick Pond, a small lake situated in the headwaters of the Humber River. Anadromous salmon have not been observed in these ponds.

A somewhat different situation exists when seaward migration is not prevented by physical barriers. In Newfoundland, anadromous and nonanadromous salmon often occupy the same watershed, particularly those containing large expanses of lake habitat. This is a common feature of many watersheds in Newfoundland, and both anadromous and nonanadromous salmon have been reported in Gander River (Verspoor and Cole 1989), Terra Nova River (Andrews 1966), Wing's Brook (Hutchings 1986), Gambo River (Leggett and Power 1969), North Arm River (Couturier et al. 1986), and many others. The genetic relationship among the two forms in this situation is not well understood. They may represent distinct breeding units that differ genetically.

Alternatively, anadromous and nonanadromous forms may not be reproductively isolated. In this case the two behavioral patterns probably represent intrapopulation variation in life history parameters. This would imply that little or no genetic differentiation is present and that within a given river the two forms do not necessarily belong to separate

reproductive units.

The purpose of this study was to evaluate the hypothesis that anadromous and nonanadromous salmon that occupy a Newfoundland river do not represent separate gene pools. Two approaches were adopted to test this hypothesis. In the first test, comparisons were made of developmental patterns displayed by offspring of the two forms cultured in the laboratory. Since the salmon were cultured under identical conditions it is assumed that any developmental differences observed are due to genetic differences. The second test consisted of an analysis of mitochondrial DNA (mtDNA) variation in the two forms of salmon. The observation of statistically significant differences in genotype frequencies would constitute strong evidence for reproductive isolation between the forms.

Developmental/Physiological Experiment

Wild broodstock were collected in the Gambo River system and their offspring were cultured under identical conditions. In this experiment presmolt growth rates and the incidence of postsmolt maturation were compared between the two groups. In addition, parameters associated with the parr-smolt transformation were compared. Migratory behaviour is the most obvious difference between anadromous and nonanadromous salmon. Selection pressures acting upon anadromous salmon at the time of seaward migration, and beyond, are very different

from those experienced by nonanadromous salmon during this period of the life history. Genetic differences (if any) present among the two forms might therefore be expected to relate to processes associated with anadromy. Since nonanadromous salmon never encounter the marine environment, physiological alterations concerned with the switch from hyperosmoregulatory to hypoosmoregulatory mechanisms may not occur in these fish. Anadromous salmon on the other hand must be capable of maintaining hydromineral in seawater, so physiological alterations associated with hypoosmoregulation are expected to occur during the smolting process. Parameters associated with smolting that were measured include seasonal changes in skin pigmentation (silvering), body moisture content, gill Na^+-K^+ ATPase activity, salinity tolerance, and plasma sodium and chloride levels. Size and abundance of gill chloride cells were measured in salmon sampled on one occasion near the time when seaward migration would occur.

Genetic Comparison

A more direct approach for assessment of the genetic relationship between sympatric anadromous and nonanadromous salmon was taken in the form of an analysis of mitochondrial DNA sequence variation. During the last decade mtDNA has become a valuable tool for evaluating genetic differences among closely related organisms. Prior to the widespread use of mtDNA, protein electrophoresis was the most common

molecular technique for these investigations. However this technique is not sufficiently sensitive to routinely detect genetic divergence among very closely related populations, especially in species that exhibit a low degree of genetic variation such as Atlantic salmon. Attempts to delineate fine scale population structuring in this species using protein electrophoresis have achieved little success (Stahl 1983, 1987; Davidson et al. 1989), therefore an analysis of mtDNA variation is appropriate. Animal mitochondrial genes accept mutations more rapidly than do most protein coding genes in the nuclear genome (Brown et al. 1979, 1982; Shields and Wilson 1987) and are therefore more sensitive indicators of genetic structuring. Most common mutations in mtDNA consist of transitions and small length mutations (Wilson et al. 1985a). Two factors appear to contribute to the rapid evolution of mtDNA. First, replication error and DNA lesions go unrepaired more frequently in mitochondrial genes than in nuclear genes due to an inefficient mismatch repair system. Second, the mitochondrial genome does not code for proteins that function in its own replication or in mitochondrial protein synthesis. Less accuracy is therefore required for replication of this system than for the extramitochondrial protein synthesis system (Cann et al. 1984; Wilson et al. 1985a).

Other factors contribute to the increased use of mtDNA analysis in population-level studies. Highly purified

material can be prepared using relatively simple techniques (Lansman et al. 1981). In animals the molecule is a small (approximately 16,000 bp in length) covalently closed circle specifying the sequence of 22 tRNAs, 2 rRNAs and 13 mRNAs. Polypeptides translated from the mRNAs are all involved with the energy production system of the mitochondrion. To date the complete nucleotide sequence has been determined for six vertebrates: Homo sapiens (Anderson et al. 1981), Bos taurus (Anderson et al. 1982), Mus (Bibb et al. 1981), Rattus norvegicus (Gadaleta et al. 1989), Gallus domesticus (Morais in prep.), and Xenopus laevis (Roe et al. 1985). The complete sequence has also been reported for Drosophila yakuba (Clary and Wolstenholme 1985) and partial sequences have been reported for many other species. These and other studies indicate that, within the vertebrates, gene content and arrangement are highly conserved despite the high variability of nucleotide sequence. The mitochondrial genome of vertebrates generally contains no repeated sequences, and spacer regions between genes, where present, consist of very few nucleotides. The only major nontranscribed region lies between the sequences encoding tRNAs for proline and phenylalanine. This region is often referred to as the D-loop (displacement loop) and contains the origin of replication for the heavy strand (Brown 1983).

Mitochondrial DNA appears to be inherited in clonal fashion from the maternal parent only. Gyllensten et al.

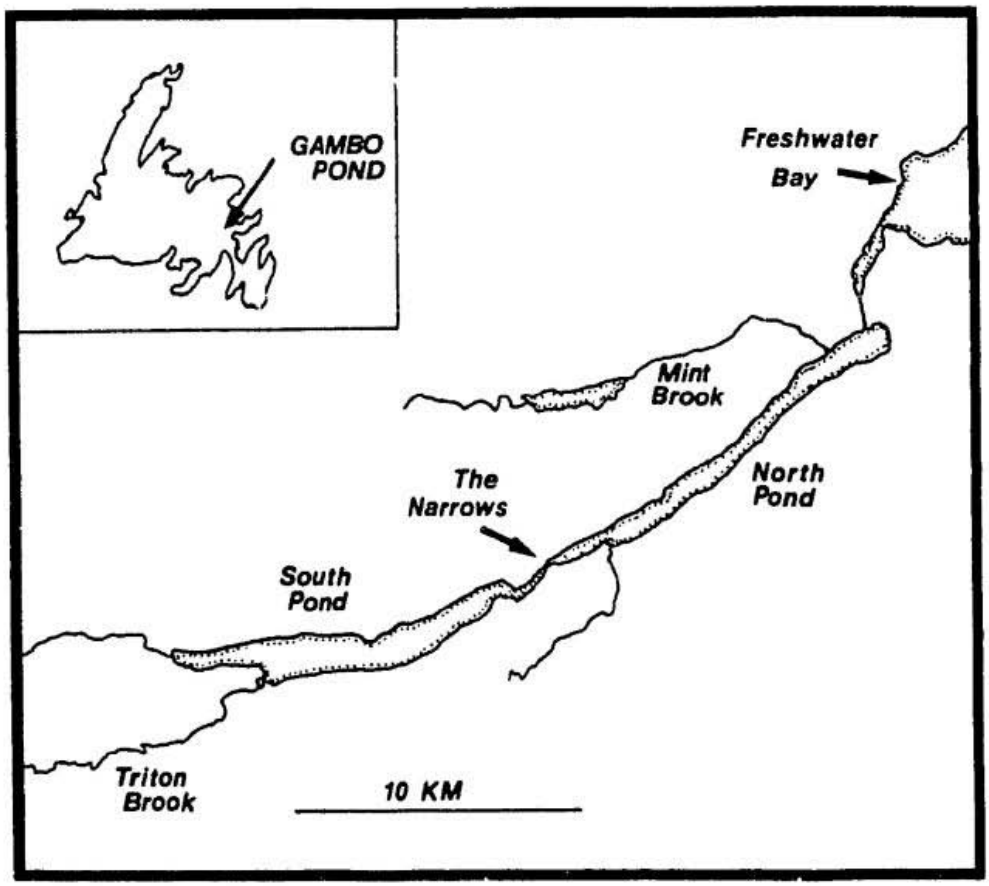
(1985) could not detect paternal inheritance in studies involving reciprocal backcrossing of female interspecific hybrid mice to males of the paternal species. As well, there appears to be no recombination in mtDNA, an attribute that makes the molecule especially useful in phylogenetic analysis.

METHODS AND MATERIALS

Study Area

Gambo Pond (48° 37'N, 54° 24'W), an oligotrophic lake in eastern Newfoundland, actually consists of two long narrow lakes separated by a short stretch of river (the Narrows). The north pond has a surface area of approximately 1100 ha and a maximum depth of 12 meters while the south pond is slightly smaller in area (950 ha) but deeper (maximum depth of 40 meters, Leggett and Power 1969). The two basins are oriented roughly along a NE-SW axis. Gambo Pond North is drained by Gambo River, which runs for approximately 2 km to Freshwater Bay. Two major tributaries empty into the system: Triton Brook enters Gambo Pond South near the south west end while Mint Brook enters near the outlet of Gambo Pond North. In addition, Riverhead Brook enters Gambo Pond South near the outlet of Triton Brook and Parson's Brook enters Gambo Pond North near the Narrows. Several small intermittent brooks enter the lakes at various other locations (Fig. 1). Three salmonine species occupy the system, including both anadromous and nonanadromous Atlantic salmon and brook charr (Salvelinus fontinalis) and a small population of nonanadromous Arctic charr (Salvelinus alpinus). In addition the system contains anadromous rainbow smelt (Osmerus mordax), American eel (Anguilla rostrata), three-spined stickleback (Gasterosteus aculeatus)

Fig. 1: Map of Gambo Pond and principle tributary streams.


GAMBO
POND
Freshwater
Bay
Mint
Brook
The
Narrows
North
Pond
South
Pond
Triton
Brook
10 KM

and nine-spined stickleback (Pungitius pungitius). During the summer months thermal stratification occurs in Gambo Pond South and nonanadromous salmon remain in this pond below the thermocline when the temperature at the surface exceeds 16°C. When temperatures are lower these salmon are distributed throughout both lakes (Leggett and Power 1969).

Salmon Culture

Adult Atlantic salmon broodstock were collected in October 1986. Information gathered from local residents and anglers encountered in the Gambo area indicated that nonanadromous salmon congregate at the outlet of Gambo Pond South during the autumn and spawn at this site. These fish were sampled using fyke nets and angling gear. Anadromous salmon were seined from pools in Triton Brook (approximately 2 km upstream from the outlet into Gambo Pond South). The two forms are easily distinguished: anadromous fish sampled were considerably larger (45.8 - 64.0 cm fork length, N=21) than nonanadromous fish (29.8 - 39.6 cm fork length, N=18). The latter are much darker in colour and have larger heads in proportion to body size. In no instance was the identity of individual fish in question. Broodstock were transported in a tank truck equipped with an aeration/recirculation system to the Ocean Sciences Centre where they were held in 2400 L circular tanks (2 m diameter) supplied with running freshwater at ambient temperature under simulated natural photoperiod.

In mid November the anadromous salmon were stripped. Three hundred eggs were collected from each of 10 females and pooled. Milt from 10 males was used to fertilize the eggs. Nonanadromous salmon were stripped three weeks later following the same procedure. Ova from the two forms were incubated in separate trays at 7°C in a temperature controlled recirculation system. Initial feeding of anadromous fry was on 15 March 1987; nonanadromous fry fed for the first time two weeks later. At swim-up, salmon were transferred to separate 1 m^2 tanks in a recirculating system (Sutterlin et al. 1983) and fed a commercially prepared salmon starter diet (Biodiet) at 15 minute intervals. Water temperature was maintained at 11°C until 10 June when the local water supply (pond near OSC) reached this level. Salmon were then transferred to new tanks with water supplied from this source on a single pass basis. On 1 September the progeny of nonanadromous parents were fin clipped (adipose); from that date onward anadromous and nonanadromous salmon were cultured as mixed groups in the same tanks. To maintain growth rates sufficient to produce a significant number of 1+ smolts, heated water (9°C) was supplied on 20 December. On 5 January 1988 the salmon were graded. Fish 10 cm and longer (fork length) were considered large enough to smoltify the following spring and were cultured separately from the smaller fish. The smolting process was monitored over the following winter and spring among the large size class salmon. On 28 April the heated

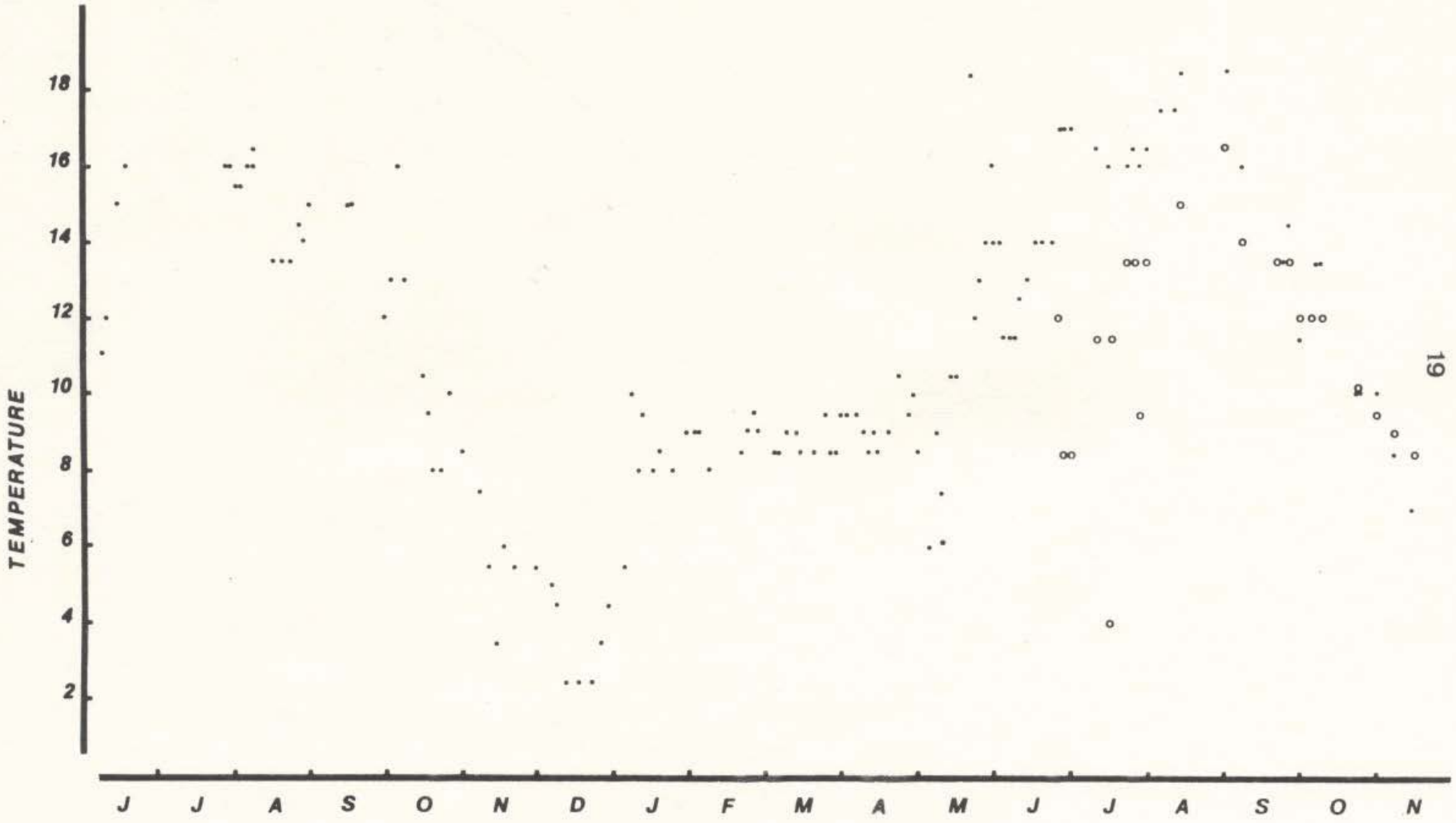
water supply was gradually reduced and the salmon were again returned to the ambient water supply in which they were maintained for the remainder of the experiment. During the rearing period salmon were maintained under simulated natural photoperiod and fed to excess with commercial salmon feed (New Life Feed, W.A. Fleming) of appropriate particle size. Fig. 2 shows temperature conditions under which salmon were cultured. No evidence of disease was observed during the rearing period and fish appeared vigorous throughout the experiment.

Growth/Condition Factor/Silvering

Periodically during the rearing experiment salmon were starved for 24 hours, anaesthetized with 2% tertiary amyl alcohol, weighed and measured. This handling did not appear to affect the fish severely: recovery was evident after 30 minutes and normal feeding behavior resumed the same day or on the following day. Condition factor was calculated using the formula $CF = 100M \cdot FL^{-3}$, where M is mass in grams and FL is fork length in centimeters. Instantaneous growth rate was calculated using the formula:

$$IGR = (\ln X_{T2} - \ln X_{T1})\ (T2 - T1)^{-1},$$

Fig. 2: Seasonal temperature conditions under which anadromous and nonanadromous salmon were cultured. Solid circles indicate freshwater conditions; open circles represent temperature conditions experienced by postsmolts cultured in seawater.


TEMPERATURE
18
16
14
12
10
8
6
4
2
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
O
N

where X is mean fork length at times T2 and T1, and T2 is later than T1.

Development of smolt coloration was also monitored on the occasions when the salmon were weighed and measured according to a modification of the method of Johnston and Eales (1967). Each fish was classified as either unsilvered (parr marks distinct, little or no silvering), partially silvered (parr marks partially obscured by silver pigment) or highly silvered (parr marks completely or almost completely obscured by silver pigment, fins darkened).

Sampling

Beginning on 7 February 1988, cultured salmon were periodically sampled to measure seasonal patterns of moisture content, branchial Na^+-K^+ ATPase activity, chloride cell development and plasma Na^+ and Cl^- concentrations. Fish were randomly netted from rearing tanks and quickly killed with a blow to the head. They were weighed, measured, the tails cut off and blood samples were collected from the severed caudal blood vessels into heparinized tubes. A portion of the first right gill arch and filaments was fixed in Bouin's fixative and the remainder of the gill system was removed and washed in ice cold 250mM sucrose, 5mM EDTA. The gills were then immersed in fresh sucrose-EDTA and rapidly frozen in a dry ice/ethanol bath. Frozen gill samples were stored at -70° for

a maximum of 48 hours before Na^+-K^+ ATPase activity was determined. The carcass was then opened along the midventral line, sexed and dried to a constant mass at 80°C to determine moisture content.

Na^+-K^+ ATPase Activity

The frozen gills were thawed and blotted dry. Filaments were trimmed from the arches, suspended in ice cold sucrose-EDTA (36 $mg \cdot mL^{-1}$) and homogenized using a motor powered teflon pestle. Na^+-K^+ ATPase activity (ouabain-sensitive) of the homogenate was determined using the technique described by Johnston and Saunders (1981) and Birt and Green (1986). A 0.2 mL aliquot of tissue homogenate was added tc 0.1 mL 1000mM NaCl, 200mM KCl solution and 0.5 mL 200mM Tris-HCl (pH 7.6). A second aliquot was added to another tube containing 0.1 mL 1000mM NaCl, 200mM KCl,2mM ouabain and 0.5 mL 200mM Tris-HCl (pH 7.6). The reaction was started with the addition of 0.2 mL 30mM ATP (disodium salt), 25 mM $MgCl_2$ and stopped exactly 10 minutes later by adding 4 mL cold 1% ammonium molybdate, 40 $mg \cdot mL^{-1}$ ferrous sulfate solution prepared in 1.15N H_2SO_4. The reactions were incubated at 37°C, then centrifuged at 750xg for 5 minutes after addition of the stop solution. The optical absorbance of the supernatant was read at a wavelength of 700 nM. Phosphate standards were prepared using KH_2PO_4.

Protein concentration was measured using a modification of the Lowry method (Hartree 1972) using bovine serum albumin

as a standard. Na^+-K^+ ATPase activity was calculated as the difference between activities in the reaction mixture containing ouabain and that without ouabain, and is expressed as micromoles inorganic phosphate liberated/mg protein/hour (μmol P_i·mg protein^{-1}·hr^{-1}). All protein and enzyme activities were determined in triplicate.

Salinity Challenge

Salinity challenge tests were carried out on two occasions during the rearing period: 9 April and 4 June, 1988. Ten salmon from each stock were placed directly in aerated seawater (32 ppt) in a tank measuring 120 x 60 x 45 cm at a temperature of 10°C. Mortalities were recorded over 96 hours.

Chloride Cells

Fixed gill filaments were dehydrated in an ethanol series, cleared and embedded in paraffin. Sections were cut longitudinally (5 μm thick) and stained with hematoxylin and eosin. Chloride cells were identified as large eosinophilic cells located most often at the bases of the secondary lamellae. For each sample, counts were made of chloride cells associated with 25 lamellae and abundance is expressed as the number of cells per lamella. The mean chloride cell length in each fish was estimated by measuring 20 cells (sectioned through the nucleus) from the basal to apical surfaces using a calibrated ocular micrometer.

Post-Smolt Maturation

On 15 June 1988 the remaining anadromous and nonanadromous salmon from the large size class were divided among two 2400 L circular tanks (2 m diameter) supplied with freshwater at ambient temperature. Salmon in one tank were then acclimated to ambient temperature seawater over a 7-day period by gradually increasing the flow rate of seawater while reducing the flow rate of freshwater. One tank therefore contained seawater-acclimated anadromous and nonanadromous salmon, while the other contained salmon of both types in freshwater. The fish were cultured throughout the summer under simulated natural photoperiod. They were fed twice daily to satiation with either a dry commercial ration (New Life Feed, W.A. Fleming) or with a moist pellet prepared on site from capelin meal (48%), capelin (35%), middlings (10%), capelin oil (5%) and vitamin mix (1.2 %). On 15 November all salmon in each group were killed, sexed, and classified as either mature or immature by visual inspection of the gonads.

Mitochondrial DNA

Mitochondrial DNA was prepared from adult salmon collected within the Gambo River system. Salmon previously transported to the Ocean Sciences Centre and used as broodstock were used as a source of mtDNA. In addition to these fish, mtDNA was prepared from 10 adult nonanadromous salmon angled from Gambo Pond South in May 1987 and from 8 anadromous smolts captured in a fyke net near the outlet of Gambo Pond North in June 1986. Preparation of mtDNA was carried out according to two methods: the alkaline extraction method described by Palva and Palva (1985) and density gradient centrifugation.

The alkaline extraction method does not rely on isopycnic centrifugation for mtDNA purification. Crude mitochondrial preparations were made by suspending chopped liver, heart and kidney tissue in ice cold buffer containing 250mM sucrose, 10mM Tris-HCl (pH 7.0), 5mM EDTA (1-2 g tissue·mL^{-1} buffer). Tissue was then homogenized with a teflon Potter-Eljhem homogenizer with a clearance of 0.3 mm. The homogenate was then spun in a refrigerated centrifuge (4°C) at 700xg for 6 minutes. The pellet, containing nuclei and cell debris was carefully separated and discarded, and the mitochondria-containing supernatant recentrifuged. The resulting supernatant was then centrifuged at 10,000xg for 20 minutes. The mitochondrial pellet was resuspended in fresh buffer, pelleted again and resuspended in 200 μL buffer containing

50mM glucose, 25mM Tris-HCl (pH 8.0), 10mM EDTA. To this suspension was added 400 μL 200mM NaOH, 1% SDS to lyse the mitochondria. This mixture was mixed gently and held on ice for five minutes and then 300 μL 3M potassium acetate (pH 4.8) was added. After gentle mixing the mixture was placed for 2 minutes at -70°C, then centrifuged for 10 minutes at 10,000xg. To 750 μL of the supernatant was added 450 μL isopropanol and tubes were placed at -70°C for 5 minutes to precipitate DNA. The tubes were then microfuged (10,000xg) for 20 minutes, decanted, washed with 70% ethanol and desiccated under vacuum. The pellet was resuspended in 100 μL TE (10mM Tris-HCl pH 8.0, 1.0 mM EDTA), treated with RNase (boiled), phenol extracted twice and extracted with chloroform, isoamyl alcohol (24:1) to remove residual phenol. The DNA was then precipitated with ethanol, desiccated under vacuum and finally suspended in 100 μL TE (Palva and Palva 1985).

To prepare highly purified mtDNA, crude mitochondrial pellets were prepared as described above and resuspended in 3 mL 100mM NaCl, 50mM Tris-HCL pH 8.0, 1mM EDTA. Mitochondria were lysed by adding 200 μL 20% SDS. The volume was adjusted to 8 mL with TE and 8.0 g solid CsCl was added and mixed. When fully dissolved 800 μL ethidium bromide (10 $mg \cdot mL^{-1}$) was added and the solution centrifuged in a Beckman 50Ti rotor for 36-48 hours at 45,000 RPM. The lower band, visualized under ultraviolet illumination, contained supercoiled mtDNA and was removed by side puncture. Ethidium bromide was removed by

repeated extraction with water-saturated isoamyl alcohol and the CsCl removed by extensive dialysis against TE (see Lansman et al. 1981).

A second method employed for preparation of highly purified material followed the method of Carr and Griffith (1987). The crude mitochondrial pellet prepared from 1-2 g of tissue was resuspended in a total volume of 2.4 mL TE. Mitochondrial lysis was achieved with addition of 600 μL 10% SDS (freshly prepared). Contaminating nuclear DNA was precipitated with the addition of 500 μL 7M CsCl; the solution was mixed gently and held on ice for at least 60 minutes. After remixing the solution was centrifuged at 17,500xg for 10 minutes at 5°C. To 2.9 mL of recovered supernatant was added 600 μL 2.0 $mg \cdot mL^{-1}$ propidium iodide (made up in TE) and the density adjusted to 1.58 $g \cdot mL^{-1}$ by adding 2.52 g solid CsCl. The resulting solution was divided among duplicate 2 mL quick-seal tubes and centrifuged in a Beckman TL100 benchtop ultracentrifuge at 100,000 RPM (436,000xg) for at least 5 hours (usually 12 hours). Two bands were visualized under ultraviolet illumination; the lower was collected by bottom puncture. The mtDNA-containing fractions from duplicate tubes were pooled and recentrifuged. The lower band containing highly purified mtDNA was collected, the CsCl removed by extensive dialysis against TE and intercalated propidium iodide removed by dialysis against an activated cation exchange resin (Biorad AG 50W-X8, 1-200 mesh) suspended in TE.

Purified mtDNA samples were digested with each of 18 restriction endonucleases according to manufacturer's directions. Restriction fragments from samples containing relatively large amounts of mtDNA and generated by enzymes yielding only large fragments were separated in 0.8% agarose and visualized directly by staining gels with ethidium bromide (1 $\mu g \cdot mL^{-1}$) followed by ultraviolet illumination.

End Labelling

In most instances restriction fragments were end labelled according to the following adaptation of the protocol of Brown (1980). Ten ng (or less) mtDNA were digested to completion in a volume of 15 μL. To the reaction mixture was added 2 μL of a cocktail containing 65 μM dGTP, dATP and dTTP, 400 mM Tris-HCl pH 7.5, 80 mM $MgCl_2$, 0.65 mM dithiothreitol, 0.5 units DNA polymerase (Klenow fragment) and 1 μCi [α-^{32}P]dCTP (3000 $Ci \cdot mMol^{-1}$, Amersham). This mixture was incubated at room temperature for 10 minutes. Labelled DNA fragments were precipitated by the addition of 3 μL tRNA (19 $mg \cdot mL^{-1}$), 40 μL sodium acetate (3 M, pH 5.8), 350 μL water and 900 μL cold 95% ethanol. Tubes were incubated at -70°C for 20 minutes, centrifuged (10,000xg) for 5 minutes, decanted, the pellets were washed 3 times with cold 70% ethanol, desiccated under vacuum and resuspended in 20 μL of buffer containing 50% glycerol and bromophenol blue. Resuspended fragments were loaded on verticle 1-1.5% agarose gels made up in TEA buffer

(40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.4). After electrophoresis the gels were dried and autoradiographed overnight at -70°C with or without intensifying screens.

Southern Blotting

Some mtDNA samples prepared by the alkaline extraction method were contaminated with nuclear DNA resulting in unacceptable levels of background when restriction fragments were visualized by end labelling. Restriction fragments generated from these samples were visualized by hybridization to ^{32}P-labelled capelin (Mallotus villosus) or salmon mtDNA followed by autoradiography. Fragments were electrophoresed in horizontal agarose slab gels, denatured during two 30-minute washes in 1.5 M NaCl, 0.5 M NaOH (250 mL per wash) and transferred to nylon membranes (Hybond N, Amersham; see Maniatis et al. 1982). Membranes were baked for 2-4 hours at 80°C and prehybridized overnight in a volume of 10 mL at 42°C. Prehybridization solution contained 50% formamide, 0.2% SDS, 5 X SSC (1 X SSC contains 150 mM NaCl, 15 mM trisodium citrate pH 7.0) and 5 X Denhardt's solution (1 X Denhardt's solution contains 0.02% Ficoll, 0.02% polyvinylpyrrolidone and 0.02% BSA). Hybridization was carried out under the same conditions in a volume of 5 mL per membrane. Highly purified mtDNA prepared from ripe capelin ova by banding twice in CsCl density gradients was used as probe. Probe was ^{32}P-labelled by nick translation to specific activities typically greater

than 1.5 X 10^7 dpm·μg^{-1}, denatured by boiling for 10 minutes and rapidly added to the prehybridization mixture. In most instances sufficient probe was added to provide 3 x 10^6 dpm per filter.

After hybridization overnight, filters were given two 15-minute washes at room temperature (with gentle shaking) in 250 mL 2 X SSC, 0.1% SDS followed by two 30-minute washes at 60°C in 0.1 X SSC, 0.1% SDS. Autoradiography was then carried out at -70°C using intensifying screens.

Statistical Procedures

Statistical comparisons of means were made using Student's t-test or analysis of variance. Chi-square tests of independence were used to compare frequencies of several variables within and among salmon types. In cases where expected cell values were less that 5, Yates' correction was employed (Bailey 1959). For statistical comparison of mtDNA genotype frequencies between anadromous and nonanadromous salmon, the randomized chi-sqare test was performed on unpooled data as described by Roff and Bentzen (1989).

RESULTS

Growth/Condition Factor

At swim-up, progeny of nonanadromous parents were significantly longer than progeny of anadromous parents (t_{43}=14.73, $p<0.001$, Table 1). The latter reached the swim-up stage approximately two weeks before the nonanadromous fry presumably due to the different spawning dates. By 2 August the anadromous fry had overtaken the nonandromous fry in mean fork length; instantaneous growth rates indicate more rapid growth among the anadromous fry during this period. Mean fork length of anadromous fry was greater than that of nonanadromous fry on 28 August (t_{84}=2.81, $p<0.01$) and on 7 November (t_{610}=2.16, $p<0.05$).

Condition factor generally decreased from 2 August until 7 November. During this period condition factors were very similar although slightly lower among progeny of anadromous salmon on 3 October (t_{542}=3.8, $p<0.001$) and 7 November (t_{632}=2.45, $p<0.05$).

At the time of grading (5 January 1988) 132 of 296 (45 percent) anadromous salmon were 10 cm in length or larger while 128 of 339 (38 percent) nonanadromous salmon had attained this length. There was no significant difference

Table 1: Mean fork length (FL), mass, condition factor (CF), instantaneous growth rates (IGR) and sample sizes (N) of cultured anadromous (above line) and nonanadromous (below line) salmon from swim-up stage to the end of the smolting period. For dates after 5 January, entries apply only to large grade salmon (> 10 cm fork length on this date). Standard deviations are shown in parentheses.

Date	FL (cm)	Mass (g)	CF	IGR	N
1987					
2 Apr	2.5 (1.12)				23
2 Aug	5.8 (0.58)	2.4 (0.82)	1.20 (0.07)	0.688	50
28 Aug	7.6 (0.89)	5.3 (2.21)	1.17 (0.08)	1.026	50
3 Oct	8.7 (1.01)	7.5 (2.92)	1.09 (0.06)	0.384	306
7 Nov	9.8 (1.14)	11.1 (4.47)	1.13 (0.07)	0.344	300
1988					
5 Jan	10.9 (0.88)	13.5 (4.17)	1.04 (0.07)		132
20 Feb	11.0 (1.02)	14.2 (5.64)	1.03 (0.07)	0.034	130
9 Apr	12.6 (1.23)	24.4 (8.61)	1.18 (0.08)	0.331	107
7 May	13.9 (1.40)	34.6 (12.28)	1.23 (0.08)	0.364	94
5 Jun	16.3 (1.69)	58.0 (19.61)	1.30 (0.08)	0.537	81
22 Jun	17.9 (1.59)	82.0 (22.71)	1.39 (0.07)	0.568	58
1987					
2 Apr	2.9 (0.12)				45
2 Aug	5.4 (0.48)	1.9 (0.51)	1.18 (0.10)	0.514	50
28 Aug	7.1 (0.59)	4.5 (1.24)	1.20 (0.06)	1.048	50
3 Oct	8.6 (0.88)	7.3 (2.43)	1.12 (0.11)	0.517	354
7 Nov	9.6 (1.04)	10.5 (3.68)	1.15 (0.08)	0.318	339
1988					
5 Jan	10.8 (0.70)	13.3 (3.21)	1.04 (0.06)		128
20 Feb	11.0 (0.89)	14.1 (4.50)	1.04 (0.06)	0.038	126
9 Apr	12.5 (1.00)	23.9 (6.89)	1.19 (0.09)	0.329	90
7 May	13.6 (1.13)	32.5 (9.12)	1.25 (0.08)	0.309	79
5 Jun	15.4 (1.21)	49.3 (12.41)	1.31 (0.07)	0.432	68
22 Jun	16.8 (1.49)	68.0 (19.88)	1.39 (0.07)	0.510	52

in mean fork length of anadromous and nonanadromous salmon included among the large size groups. Over the subsequent winter and early spring mean fork length did not differ significantly among groups. By 5 June however, anadromous salmon were longer than nonanadromous salmon (t_{143}=3.54, $p<0.001$) and this difference in length was also observed on 22 June (t_{107}=3.72, $p<0.001$). Instantaneous growth rates are consistent with significant size differences in June. On the three sampling occasions during May and June instantaneous growth rates were higher among anadromous than nonanadromous salmon. Elevated growth rate did not translate into lower condition factor. At no time between 5 January and 22 June did condition factors differ significantly between groups. Neither group of salmon exhibited a springtime reduction in condition factor associated with smolting. Analysis of variance revealed highly significant increases in condition factors between 20 February and 22 June in anadromous ($F_{5,596}$=239.66, $p<0.001$) and nonanadromous ($F_{5,537}$=321.36, $p<0.001$) groups.

Figs. 3 and 4 show length frequency distributions for anadromous and nonanadromous salmon on three occasions prior to grading. Length frequency distributions appeared unimodal throughout this period.

Fig. 3: Length frequency distribution of anadromous parr on three dates prior to grading.

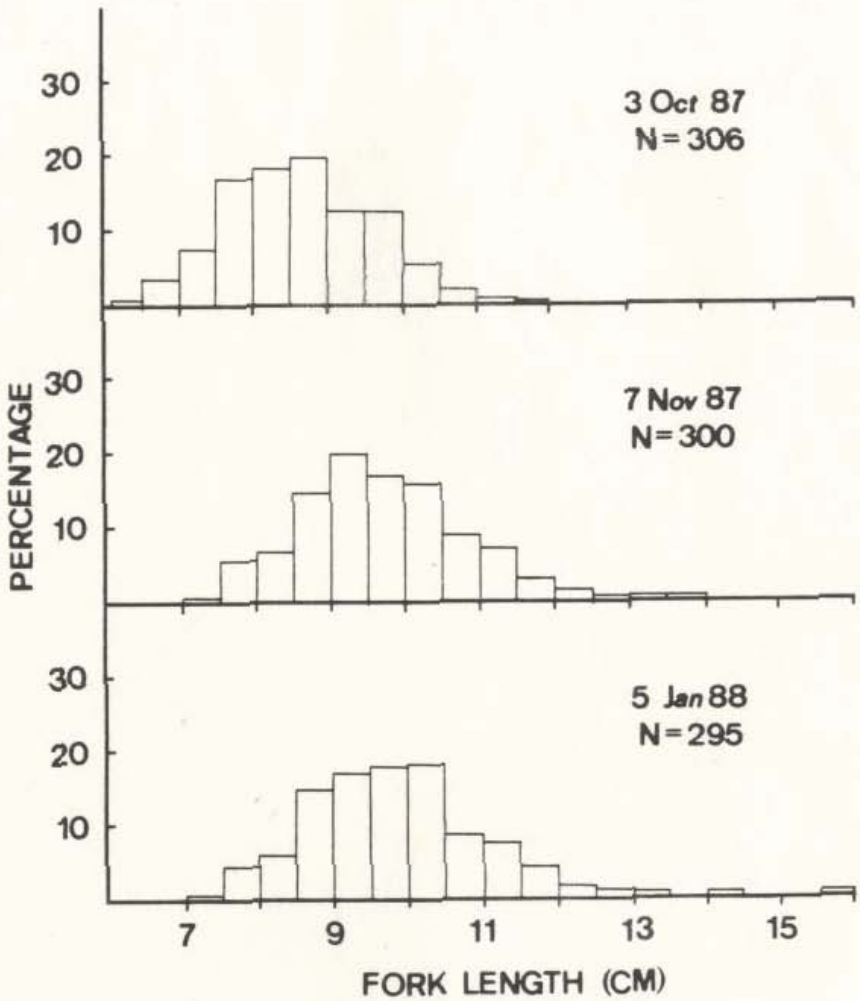
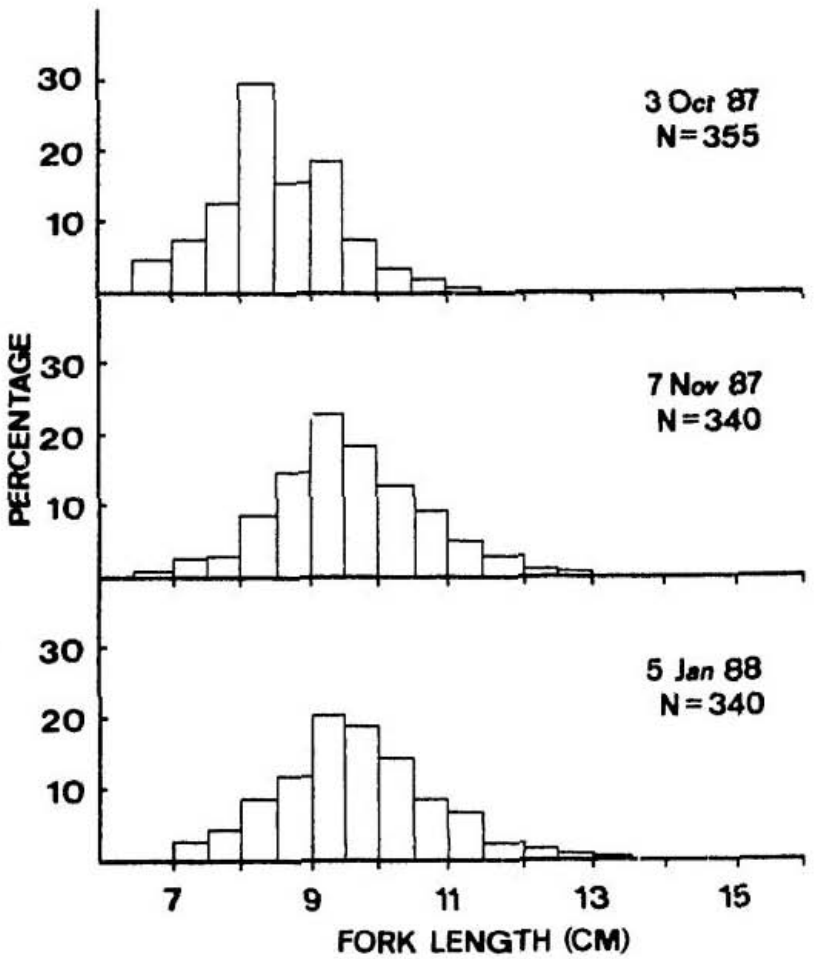

3 Oct 87
N = 306
7 Nov 87
N = 300
5 Jan 88
N = 295
PERCENTAGE
30
20
10
7
9
11
13
15
FORK LENGTH (CM)

Fig. 4: Length frequency distribution of nonanadromous salmon parr on three dates prior to grading.


30
20
10
3 Oct 87
N=355
30
20
10
7 Nov 87
N=340
30
20
10
5 Jan 88
N=340
PERCENTAGE
7
9
11
13
15
FORK LENGTH (CM)

Silvering

Table 2 presents the numbers and percentages of unsilvered, partially silvered and highly silvered salmon observed among the large size class anadromous and nonanadromous salmon on sampling dates between 9 April and 22 June. On 9 April at least 75% of salmon in both groups displayed some degree of integumentary silvering, although neither group contained a large proportion of fish with complete smolt coloration. By 3 May the proportion of unsilvered salmon had decreased and the proportion of highly silvered fish had increased among the anadromous group (x^2=10.09, df=2, p<0.01). On 7 June all anadromous salmon were classified as partially silvered or highly silvered, and the proportion of highly silvered fish had increased from 19.1% to 63% by that date. The degree of silvering was highly dependent upon date (comparing values on 3 May v.s. 7 June; x^2=40.3, df=2, p<0.001). No significant change in the frequencies of partially and highly silvered salmon was observed among the anadromous group between 7 June and 22 June.

Progeny of nonanadromous salmon also displayed a trend toward increased silvering during this time period. In contrast to anadromous salmon there was no significant increase in degree of silvering between 9 April and 3 May.

Table 2: Numbers of unsilvered, partially silvered and highly silvered salmon among the large size classes of cultured salmon during the smolting period; percentages are shown in parentheses.

	ANADROMOUS	NONANADROMOUS	
	9 April		
unsilvered	26 (24.3)	19 (21.1)	
partially silvered	75 (70.1)	70 (77.8)	n.s.
highly silvered	6 (5.6)	1 (1.1)	
	3 May		
unsilvered	14 (14.9)	12 (15.2)	
partially silvered	62 (66.0)	62 (78.5)	x^2=6.25, df=2
highly silvered	18 (19.1)	5 (6.3)	p<0.05
	7 June		
unsilvered	--	--	
partially silvered	30 (37.0)	44 (64.7)	x^2=11.26, df=1
highly silvered	51 (63.0)	24 (35.3)	p<0.001
	22 June		
unsilvered	--	--	
partially silvered	13 (22.4)	29 (55.8)	x^2=12.79, df=1
highly silvered	45 (77.6)	23 (44.2)	p<0.001

Between 3 May and 7 June however, the degree of silvering was not independent of sampling date (x^2=27.04, df=2, p<0.001). No significant increase in silvering was evident between 7 June and 22 June although the proportion of nonanadromous salmon classified as highly silvered increased from 35.3 to 44.2 percent.

Comparing anadromous and nonanadromous salmon, the degree of silvering was independent of group on 9 April. On 3 May the degree of silvering was not independent of group (x^2=6.25, df=2, p<0.05). Silvering among the anadromous group appeared to proceed more rapidly than among the nonanadromous group between 3 May and 7 June. By the latter date the degree of silvering was highly dependent upon group (x^2=11.26, df=1, p<0.001), as it was on 22 June (x^2=12.79, df=1, p<0.001).

Moisture

Seasonal patterns of total body moisture content for cultured anadromous and nonanadromous salmon are shown in Fig. 5. Comparison of mean moisture content values within and between groups was made using two-way analysis of variance (Table 3). Significant differences were detected within and among groups over the sampling period; significant interaction of factors was also detected. In both groups the observed pattern was one of decreasing seasonal values. Peak values were observed in both groups

Fig. 5: Seasonal levels of total body moisture content in cultured Atlantic salmon. Each point represents the mean of 6 salmon + 1 standard deviation. Solid line, anadromous; dashed line, nonanadromous salmon.

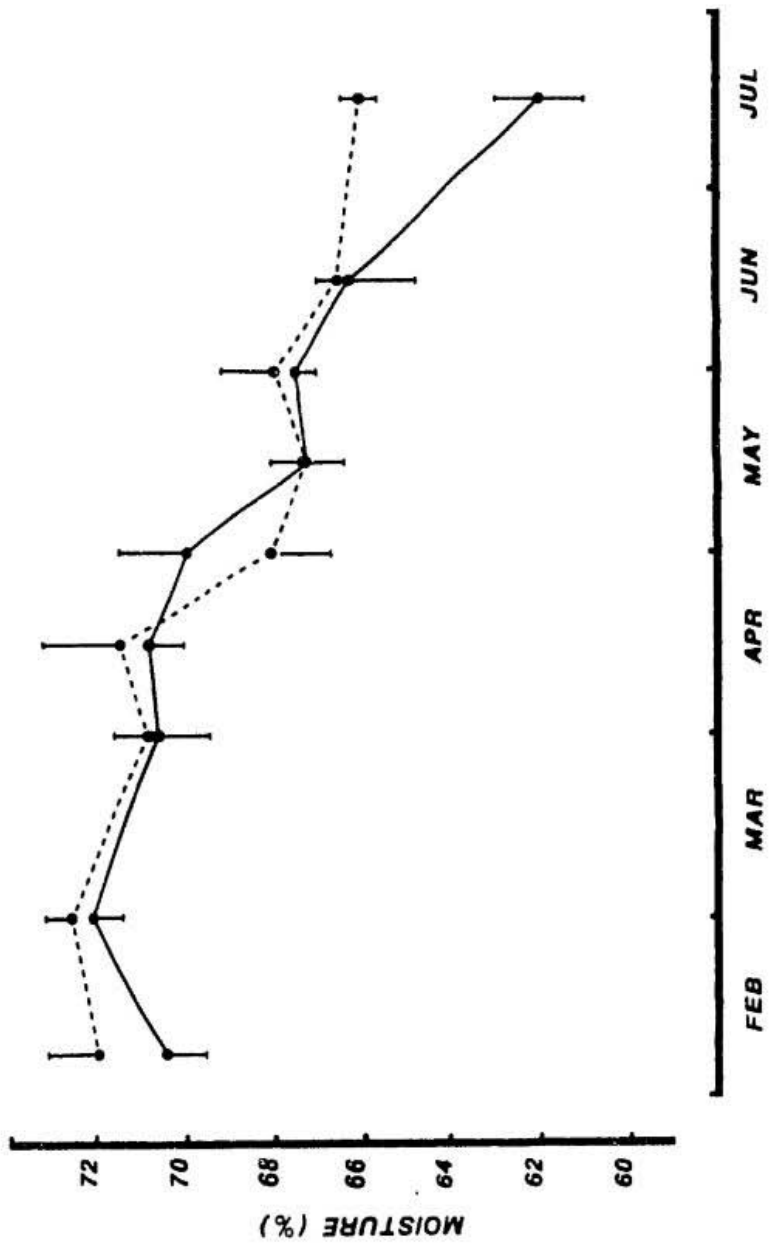

MOISTURE (%)
72
70
68
66
64
62
60
FEB
MAR
APR
MAY
JUN
JUL

Table 3. Two-way analysis of variance of seasonal mean moisture content within and between groups of cultured anadromous and nonanadromous salmon.

Source	df	SS	MS	F	p
Group	1	9.47	9.47	5.71	0.02
Date	8	714.78	89.35	53.89	0.00
Interaction	8	59.72	7.46	4.50	0.00
Error	90	149.22	1.66		

on 1 March. Among the anadromous salmon, mean moisture content was significantly reduced (relative to the value observed on 1 March) on 15 May and all subsequent dates ($p<0.01$). Significant reduction in mean moisture content among the nonanadromous salmon was evident on 1 May and on subsequent dates ($p<0.01$).

Between mid June and mid July mean body moisture content did not change significantly among nonanadromous salmon. In contrast mean body moisture fell sharply during this time among anadromous salmon ($p<0.01$).

During the interval 7 February - 15 June, mean body moisture content did not differ significantly among the two groups. A highly significant difference between groups was observed in mid July ($p<0.01$) due to lower values among the anadromous salmon.

Plasma Na^+/Cl^-

Figs. 6 and 7 present seasonal levels of plasma Na^+ and Cl^- in the cultured salmon. Two-way analysis of variance did not detect significant differences between mean values in anadromous and nonanadromous salmon on the nine sampling dates between 7 February and 15 July; interaction effects were not significant (Table 4). Significant seasonal differences in plasma Na^+ concentration within groups were noted. Mean plasma Na^+ concentration in anadromous salmon remained quite constant between 7 February and 1 May (139.8 - 142.5 meq/L). Between 1 May and 15 May mean plasma Na^+ concentration appeared to decrease, although the difference in values on these dates is not statistically significant. By 1 June mean plasma Na^+ concentration had increased from that observed on 15 May ($p<0.01$). A sharp decrease occurred between 1 June and 15 June ($p<0.01$), followed by a significant increase by 15 July ($p<0.01$).

Plasma Na^+ levels in nonanadromous salmon were very similar to those observed in the anadromous group. Mean concentration on 15 May was significantly reduced from that recorded on 1 May ($p<0.01$). A highly significant increase was observed between 15 May and 1 June ($p<0.01$), followed by a sharp decrease by 15 June ($p<0.01$). By 15 July mean plasma Na^+ concentration had risen significantly ($p<0.01$).

As was observed with plasma Na^+, mean plasma Cl^- levels

Fig. 6: Seasonal plasma Na^+ concentration in cultured Atlantic salmon. Each point represents the mean of 6 fish + 1 standard deviation. Solid line, anadromous; dashed line, nonanadromous salmon.

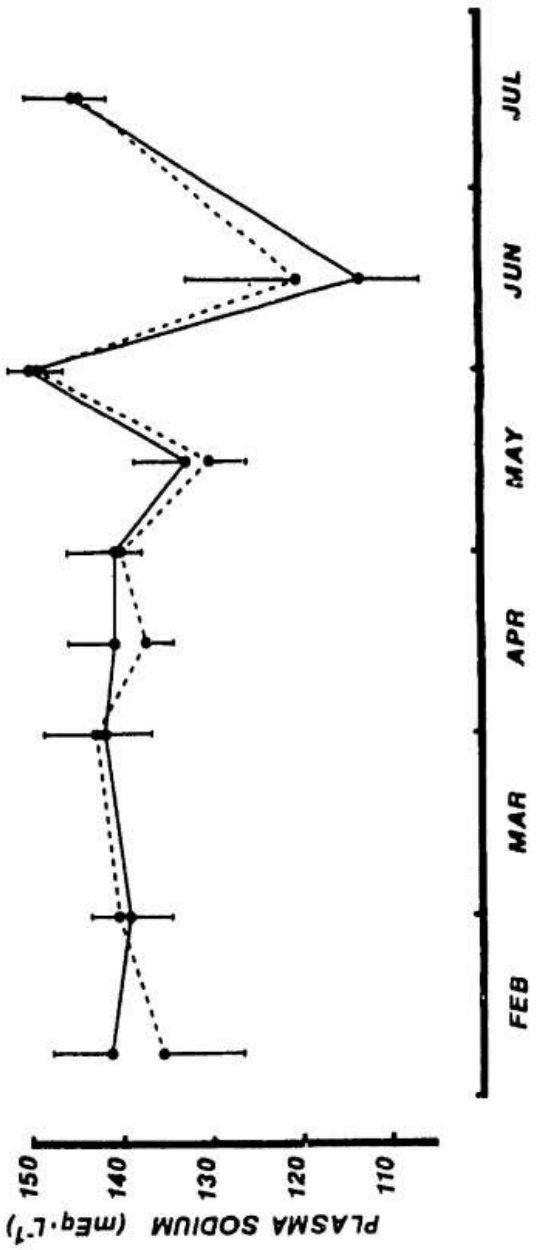

PLASMA SODIUM (mEq·L⁻¹)
150
140
130
120
110
FEB
MAR
APR
MAY
JUN
JUL

Fig. 7: Seasonal plasma Cl^- concentration in cultured Atlantic salmon. Each point represents the mean of 6 fish + 1 standard deviation. Solid line, anadromous; dashed line, nonanadromous salmon.

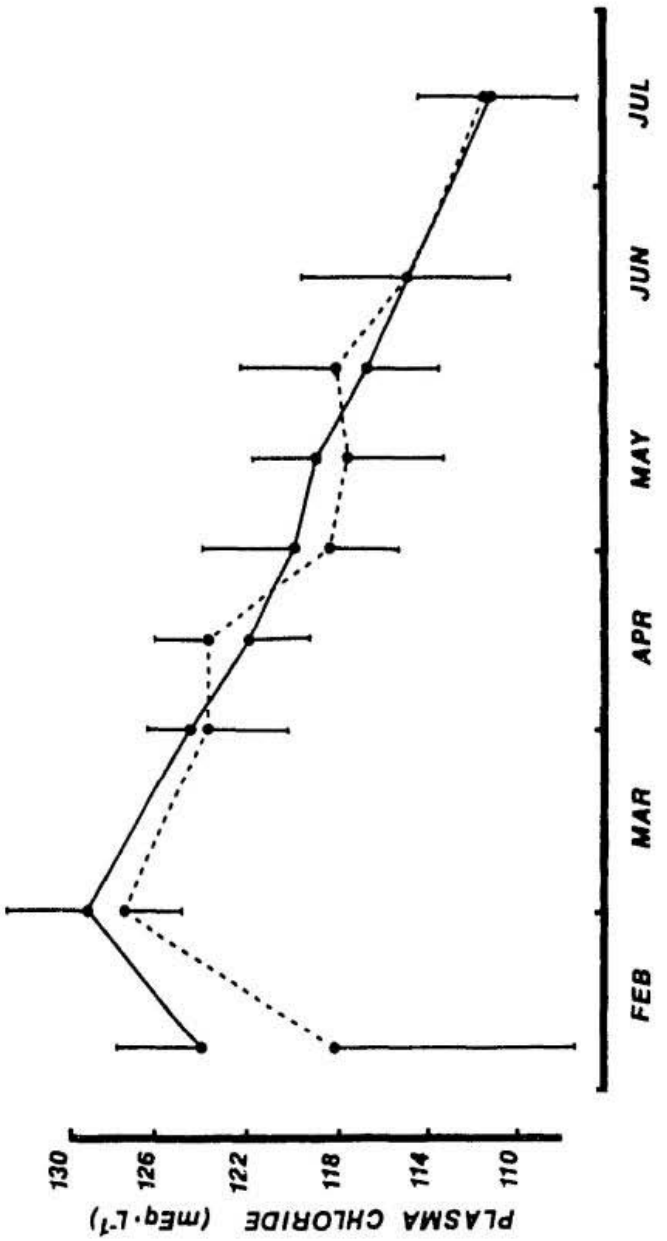

PLASMA CHLORIDE (mEq·L⁻¹)
130
126
122
118
114
110
FEB
MAR
APR
MAY
JUN
JUL

Table 4: Two-way analysis of variance of seasonal plasma Na^+ concentration within and between cultured anadromous and nonanadromous salmon.

Source	df	SS	MS	F	p
Group	1	14.88	14.88	0.31	0.59
Date	8	8534.5	1066.81	22.34	0.00
Interaction	8	290.48	36.31	0.76	0.76
Error	90	4297.00	47.74		

did not differ significantly among anadromous and nonanadromous groups on any sampling date, nor was significant interaction among factors detected (Table 5). In general, a more or less constant decrease in plasma Cl^- levels was observed over the sampling period in both groups (Fig. 7). The highest concentrations were observed on 1 March in both groups. Values were higher on this date than those observed on 7 February, although the mean plasma concentrations were not significantly higher in either group on these dates. Among the anadromous salmon a significant decrease in mean plasma Cl^- level relative to that on 1 March was observed by 1 June ($p<0.01$). Mean concentrations on all subsequent sampling dates were significantly lower than the highest seasonal level observed on 1 March. Among nonanadromous salmon, mean plasma Cl^- concentration was significantly reduced from the peak level observed on 1 March on 15 June ($p<0.01$) and 15 July ($p<0.01$).

Table 5. Two-way analysis of variance of seasonal plasma Cl^- concentration within and between cultured anadromous and nonanadromous salmon.

Source	df	SS	MS	F	p
Group	1	19.50	19.50	0.70	0.41
Date	8	2503.88	312.98	11.24	0.00
Interaction	8	129.96	16.24	0.58	0.58
Error	90	2505.67	27.84		

Salinity Challenge

Mortality was very low during salinity challenge experiments conducted on 9 April and 4 June (Table 6). During the April test only 1 of 10 anadromous salmon died while 2 of 10 nonanadromous salmon did not survive. All mortalities occurred during the fourth day of exposure to seawater. Mean fork length did not differ between the groups of test fish, however it is notable that salmon dying during the exposure period were the smallest individuals within their groups. Surviving fish at the end of the experiment appeared normal and healthy.

No mortality was observed among either group during 96 hours exposure to seawater in early June. Challenged salmon appeared perfectly healthy at the end of the experiment.

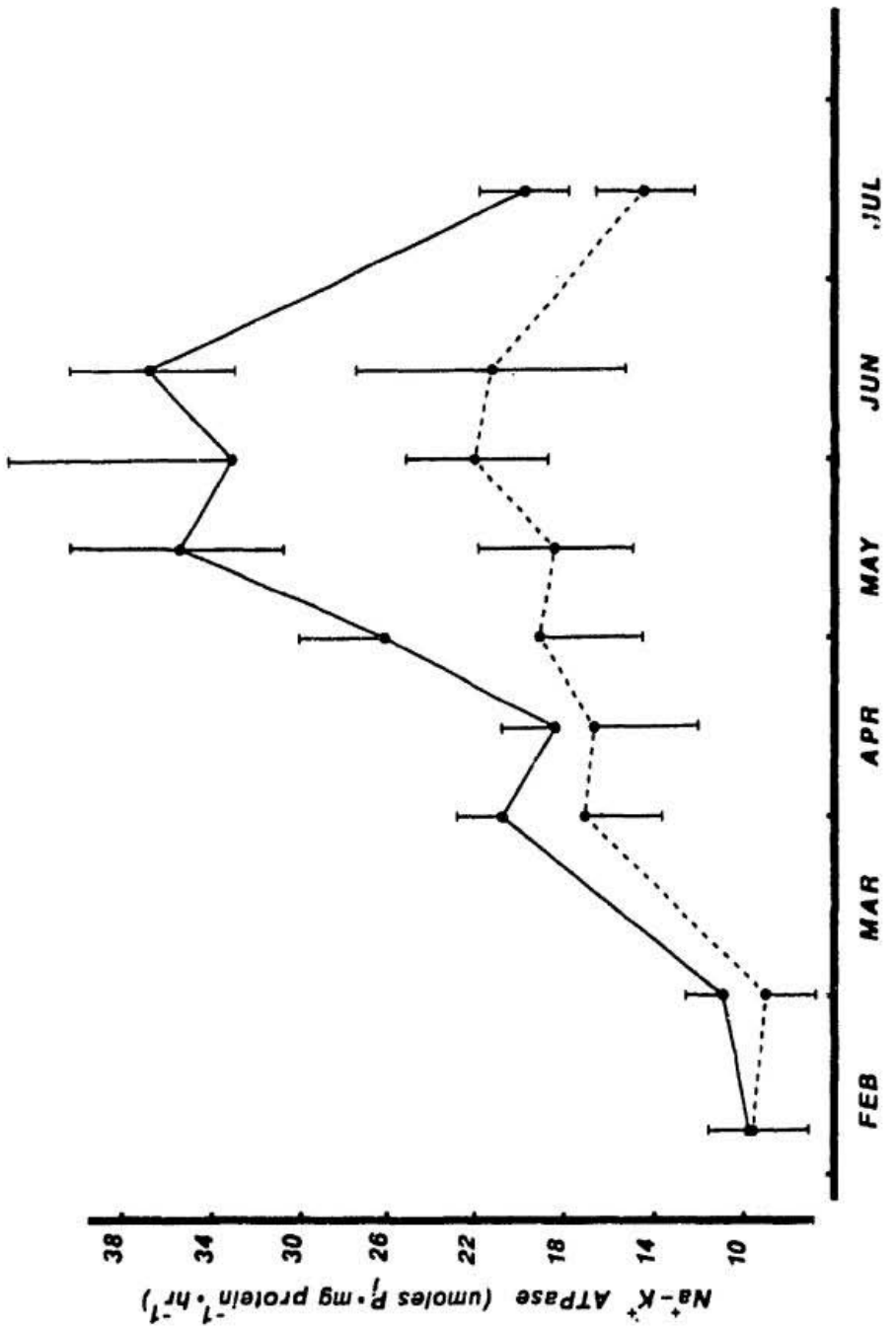
Na^+-K^+ ATPase Activity

Seasonal profiles of gill Na^+-K^+ ATPase activity are illustrated in Fig. 8. Two-way analysis of variance

Table 6: Fork length (FL) and time until death (T) for cultured anadromous and nonanadromous Atlantic salmon during salinity challenge tests. Mean fork lengths are shown at the bottom of the appropriate columns.

		9 April		4 June	
		FL(cm)	T(hr)	FL(cm)	T(hr)
anadromous		10.4	82.5	17.2	>96
		12.0	>96	18.5	>96
		11.7	>96	20.3	>96
		12.9	>96	17.0	>96
		12.6	>96	15.8	>96
		12.5	>96	16.1	>96
		11.2	>96	17.3	>96
		11.0	>96	12.7	>96
		11.8	>96	14.9	>96
		11.8	>96	15.9	>96
	$\bar{x}$	11.8		16.5	
nonanadromous		10.9	76	17.2	>96
		11.1	81	14.9	>96
		11.3	>96	16.4	>96
		12.2	>96	14.2	>96
		12.3	>96	15.1	>96
		12.8	>96	17.1	>96
		12.9	>96	13.6	>96
		12.7	>96	16.0	>96
		11.7	>96	17.2	>96
		11.2	>96	16.3	>96
	$\bar{x}$	11.9		15.8	

Fig. 8: Seasonal levels of branchial Na^+-K^+ ATPase activity in cultured Gambo salmon. Each point represents the mean of 6 fish + 1 standard deviation. Solid line, anadromous; dashed line, nonanadromous salmon.


FEB
MAR
APR
MAY
JUN
JUL
10
14
18
22
26
30
34
38
Na+-K+ ATPase (umoles Pi • mg protein-1 • hr-1)

indicates highly significant seasonal differences within and between groups as well as interaction effects (Table 7). On 7 February and 1 March both anadromous and nonanadromous groups had low mean enzyme activities typical of winter conditions. Significant induction in mean Na^+-K^+ ATPase activity occurred between 1 March and 1 May among anadromous salmon ($p<0.01$). Enzyme activity remained significantly elevated (relative to that recorded on 1 March) until 15 June ($p<0.01$).

Na^+-K^+ ATPase activity was significantly elevated among nonanadromous salmon (relative to mean value on 1 March) on

Table 7. Two-way analysis of variance of seasonal branchial Na^+-K^+ ATPase activity within and among cultured anadromous and nonanadromous salmon.

Source	df	SS	MS	F	p
Group	1	1346.20	1346.20	50.79	0.00
Date	8	5022.79	627.85	23.69	0.00
Interaction	8	923.88	115.49	4.36	0.00
Error	90	2385.62	26.51		

1 June ($p<0.01$) and 15 June ($p<0.01$). Mean seasonal activities peaked at 36.9 μmoles $P_i \cdot mg\ protein^{-1} \cdot hr^{-1}$ among anadromous salmon on 15 June. The highest mean activity observed among nonanadromous salmon was recorded on 1 June (22.2 μmoles $P_i \cdot mg\ protein^{-1} \cdot hr^{-1}$). By 15 July, mean activity had fallen sharply in anadromous salmon ($p<0.01$). Similarly,

mean activity in nonanadromous salmon decreased, although not significantly, during this period. Mean Na^+-K^+ ATPase activity was significantly higher in anadromous than nonanadromous salmon on 15 May ($p<0.01$), 1 June ($p<0.01$), and 15 June ($p<0.01$).

Chloride Cells

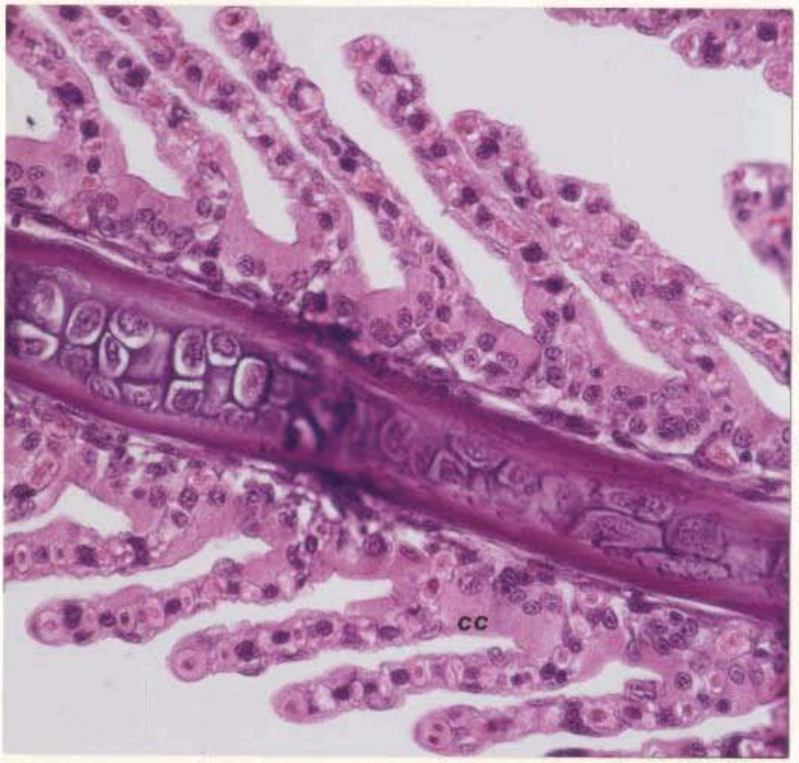
Chloride cells were obvious in gill sections from salmon sampled on 31 May. Most chloride cells were columnar in shape and were situated in the filament epithelium, although some were also observed in proximal regions of the lamellar epithelium (Fig. 9). Staining intensity did not appear to differ between anadromous and nonanadromous salmon, although mean chloride cell length was greater among the former group ($t_5=4.62$, $p<0.01$). In addition, there were more chloride cells present in the gills of anadromous salmon ($t_5=3.19$, $p<0.05$; Table 8).

Post-Smolt Growth and Maturation

Table 9 shows the proportions of mature and immature postsmolts from both groups after 146 days culture in freshwater and seawater. Sexual maturation was not evident among male or female anadromous salmon in either condition. Similarly all female nonanadromous post-smolts remained immature during this time. Maturity was evident among male nonanadromous post-smolts. Of those cultured in freshwater,

12 of 13 matured while 3 of 6 matured in seawater. Contingency analysis of the frequency of maturation among male anadromous and nonanadromous salmon (freshwater and seawater conditions combined) indicates that the frequency of maturation is not independent of group ($x^2=26.7$, df=1, $p<0.001$). Mortality during this phase of the study (22 June -15 November) was low; one nonanadromous salmon (sex unknown) in the freshwater group did not survive. Growth rates of post-smolts appeared to be greater among those cultured in freshwater than those cultured in seawater. The following groups displayed significantly greater fork lengths after culture in freshwater than after culture in seawater: male anadromous ($t_{18}=2.18$, $p<0.05$), female anadromous ($t_{24}=2.91$, $p<0.01$) and female nonanadromous ($t_{13}=3.65$, $p<0.005$). There were insufficient immature male nonanadromous post-smolts for statistical comparison, however the single male in freshwater had a greater fork length than the three immature males cultured in seawater. Fork lengths of sexually mature nonanadromous males cultured in seawater versus freshwater were not significantly different. In general comparable groups of anadromous and nonanadromous fish did not differ significantly in fork length at the end of the experiment except in one case: female nonanadromous salmon were significantly longer than female anadromous fish cultured under the same conditions

Fig. 9: Light micrograph of sectioned gill tissue sampled from cultured anadromous (top panel) and nonanadromous (bottom panel) Atlantic salmon on 31 May 1988. Chloride cells (cc) are identified as large eosinophilic cells situated primarily at the bases of the lamellae in the filament epithelium.


cc

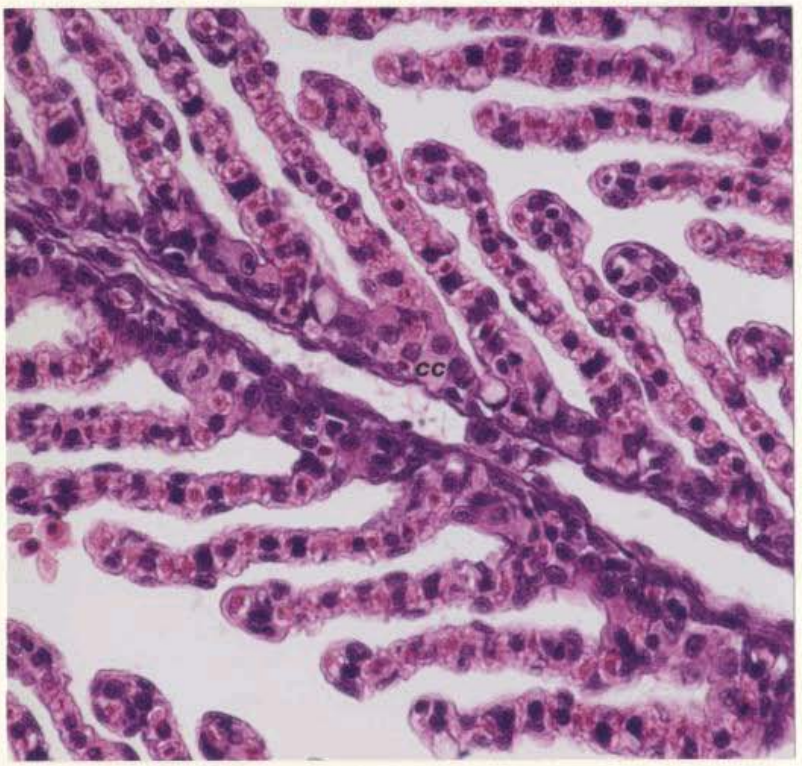

cc

Table 8: Mean number of chloride cells per gill lamella and mean length of chloride cells from cultured anadromous and nonanadromous salmon sampled on 31 May. Standard deviations are shown in parentheses.

	ANADROMOUS	NONANADROMOUS	
Chloride cells per lamella	2.7 (1.05)	1.3 (0.23)	$t_5=3.19$ $p<0.05$
Chloride cell length (μm)	16.0 (1.23)	13.5 (0.37)	$t_6=4.72$ $p<0.005$
N	6	5	

Table 9: Mean fork lengths of anadromous amd nonanadromous postsmolts before and after 146 days culture in either freshwater or seawater. The numbers of maturing and immature salmon in each condition are also shown. Sample sizes appear in parentheses.

		ANADROMOUS		NONANADROMOUS	
		SW	FW	SW	FW
22 Jun		18.5 (26)	17.7 (23)	16.5 (20)	17.2 (22)
	mature	--	--	18.3 (3)	18.5 (12)
	immature	21.5 (12)	22.9 (9)	20.5 (3)	22.3 (1)
15 Nov					
	mature	--	--	--	--
	immature	20.8 (14)	22.3 (14)	21.1 (14)	24.5 (8)

(t_9=2.73, $p<0.05$).

Growth rates among anadromous and nonanadromous post-smolts were rather low compared to those reported in other studies. For example, Saunders and Henderson (1969) found that cultured salmon reached a mean length of 31 cm by November, following approximately six months of post-smolt growth. Similarly, Allen et al. (1972) reported that post-smolts captured in the Bay of Fundy in mid August had attained a mean length of approximately 29 cm. The reasons for the relatively small length increments observed in the present study are not known. Salmon appeared healthy and mortality was very low during the post-smolt period.

Mitochondrial DNA

The restriction endonucleases used in this survey and the sizes of fragments generated by each enzyme are shown in Table 10. All enzymes cleaved Atlantic salmon mtDNA at least once. The number of restriction fragments produced per restriction enzyme ranged from 1 - 11. A total of 57 restriction sites were detected. Figs. 10 - 12 show representative examples of mtDNA fragment patterns generated by the five enzymes that revealed sequence variation and by two enzymes that did not reveal variation. By summing the sizes of fragments generated by various enzymes it is estimated that the mitochondrial genome of this species contains approximately 16,700 base pairs. Double digests were used to construct a partial

restriction site map using the single BglII site (pattern A) as the reference point (Fig. 13). Also included in this map are some sites reported by Bermingham et al. (1988) but not mapped in this study. Sequence analysis of the cloned 2.1 kb XbaI fragment revealed that it contains genes for ATPase 6 and cytochrome oxidase III genes (Davidson et al. 1989b). Using this information the locations of other genes relative to the restriction site map can be predicted assuming the gene content and arrangement are the same as in the mitochondrial genome of humans (Anderson et al. 1981) and several other vertebrates. Five restriction endonucleases produced variable fragment patterns. Variant patterns for each of these enzymes could be accounted for by the loss or gain of single sites. Site gains and losses are assumed to be due to single-base substitutions. Evidence for heteroplasmy or length polymorphism was not observed. Restriction fragment patterns generated by individual enzymes were assigned upper case letter designations. Each salmon can then be assigned a composite mtDNA genotype by combining information from each enzyme. Four such genotypes were observed among the 71 Atlantic salmon screened with all 18 restriction enzymes (Table 11). Estimates of the degree of nucleotide sequence divergence between genotypes were calculated separately from restriction fragment patterns generated by enzymes with 6-base recognition sequences and by HincII (16/3-base

Table 10: Fragment patterns generated by restriction endonucleases used to screen anadromous and nonanadromous Gambo salmon mtDNA.

1 Bgl II		2 BstE II		3 Dra I		4 Hinc II	
A	B	A	B	A	B	A	B
16.7	15.8	16.7	10.2	10.2	8.5	4.1	4.1
	0.86		6.5	6.0	6.0	3.45	3.45
				0.65	1.7	2.4	2.4
					0.65	1.8	1.8
						1.7	1.15
						0.95	0.95
						0.75	0.75
						0.65	0.65
						0.60	0.60
						0.56	0.56
							0.55

5 Pvu II		6 BamH I	7 Bgl I	8 EcoR I	9 EcoR V	10 Hind III
A	B	A	A	A	A	A
6.8	9.3	10.1	5.5	7.9	13.1	9.0
3.5	3.5	6.6	5.5	4.9	1.7	4.2
2.6	2.6		4.3	4.0	1.6	3.5
2.5	1.4		1.2			
1.4						

11 Hpa I	12 Pst I	13 Sac I	14 Sac II	15 Sal I	16 Sma I
A	A	A	A	A	A
7.0	12.4	16.7	15.0	16.7	16.7
4.5	4.0		1.7		
3.6	0.3				
1.7					

17 Xba I	18 Xho I
A	A
5.1	16.7
3.7	
3.4	
2.4	
2.1	

Fig. 10: Autoradiogram of end-labelled salmon mtDNA restriction fragments produced by EcoRV (lanes 1-4), Eco RI (lanes 5-8) and HincII (lanes 9 and 10). Fragment sizes were determined using HindIII-digested lambda phage DNA as standards and are indicated in kilobase pairs. Lanes 9 and 10 show 'A' and 'B' fragment patterns respectively.

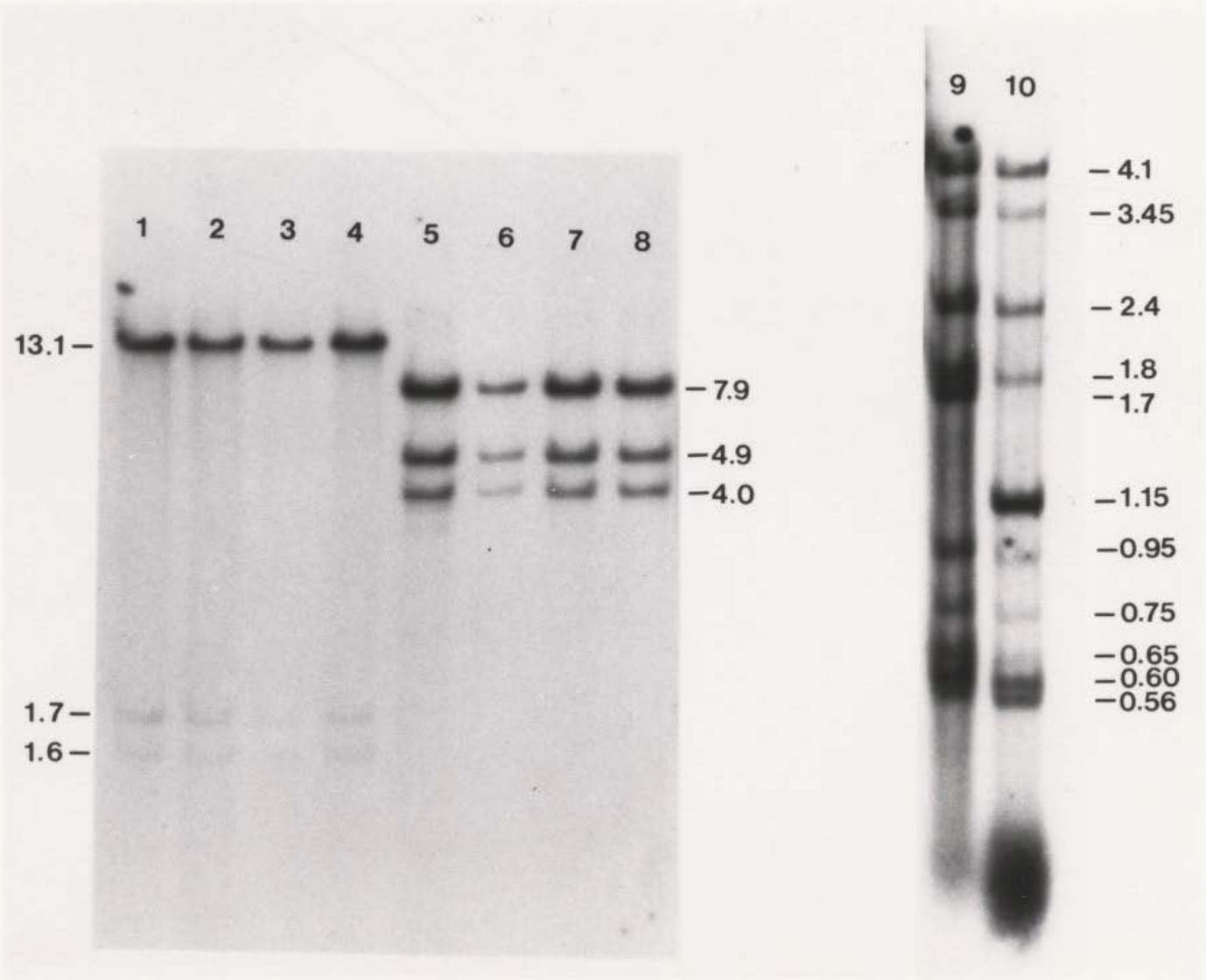

1
2
3
4
5
6
7
8
13.1
7.9
4.9
4.0
1.7
1.6
9
10
4.1
3.45
2.4
1.8
1.7
1.15
0.95
0.75
0.65
0.60
0.56

Fig. 11: Autoradiogram of variable restriction fragment patterns produced by BglII ('A' pattern, lanes 1-3; 'B' pattern, lane 4), BstEII ('A' pattern, lanes 5-7; 'B' pattern, lane 8;) and DraI ('A' pattern, lanes 9, 10 and 12; 'B' pattern, lane 11). Fragment sizes are indicated in kilobase pairs. The 10.2 kb band in lane 11 is not visible in this sample.

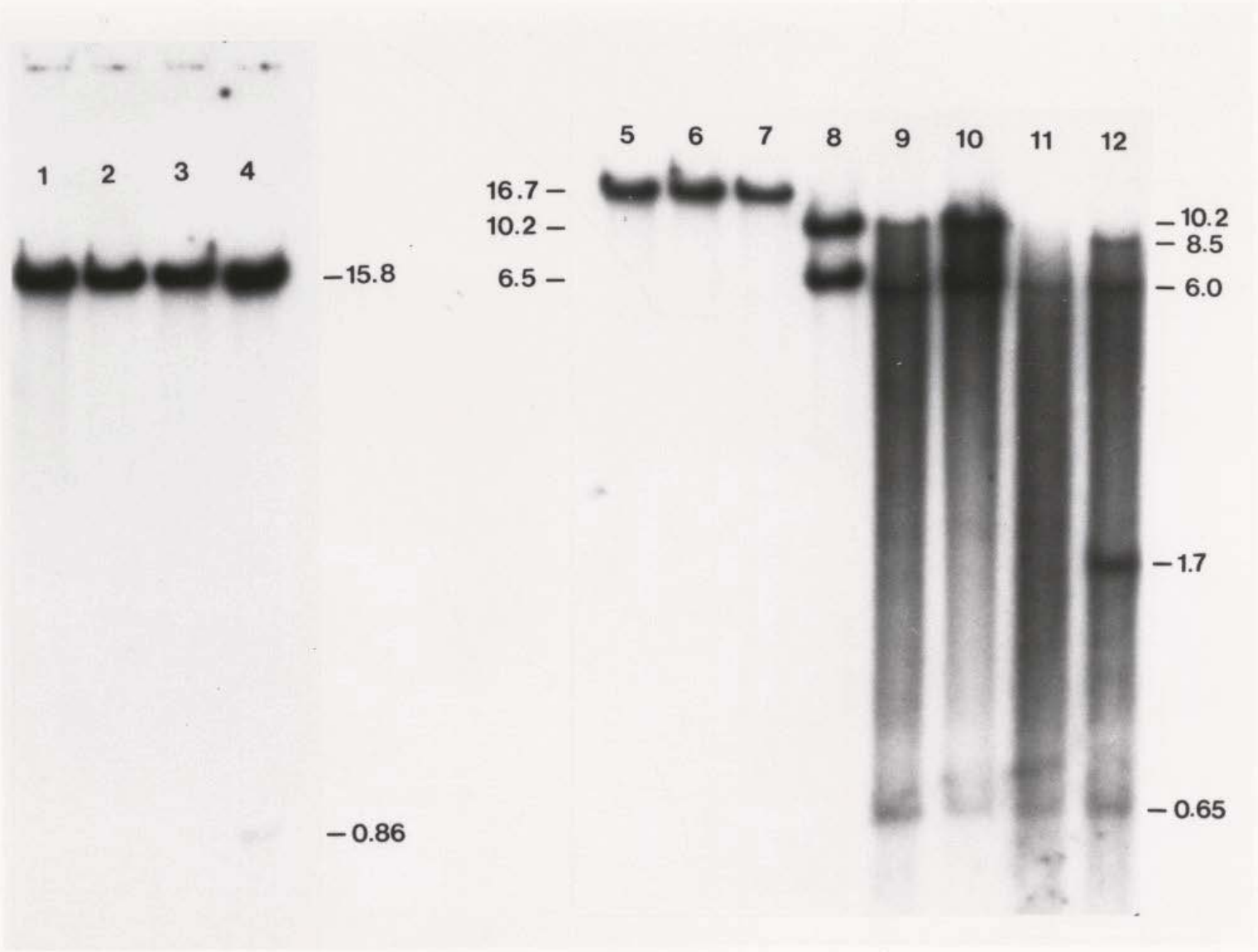

1
2
3
4
15.8
0.86
5
6
7
8
9
10
11
12
16.7
10.2
6.5
10.2
8.5
6.0
1.7
0.65

Fig. 12: Autoradiogram of variable restriction fragment patterns produced by PvuII ('A' pattern, lane 1; 'B' pattern, lane 2). Fragment sizes are indicated in kilobase pairs.

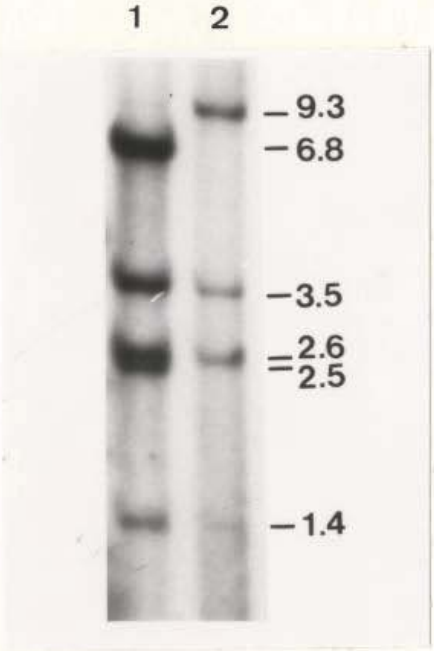
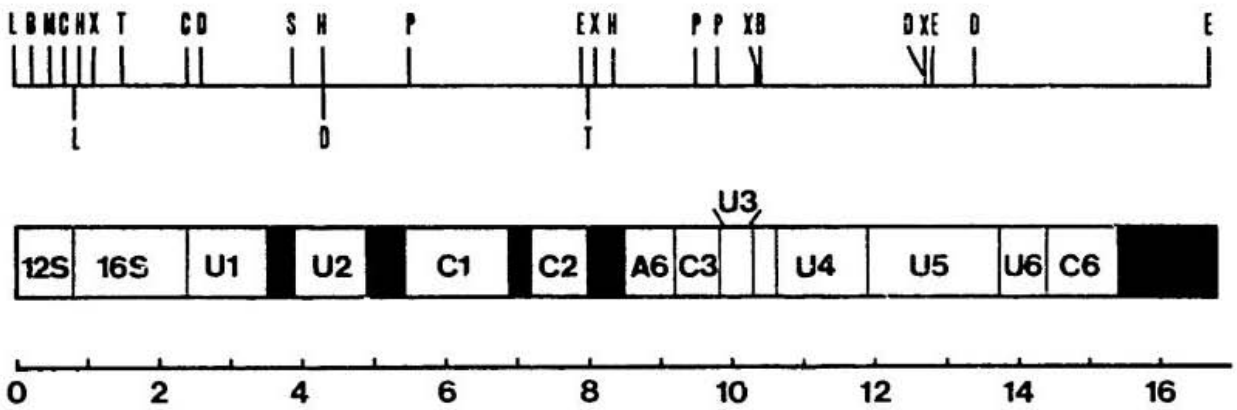

1
2
9.3
6.8
3.5
2.6
2.5
1.4

Fig. 13: Restriction site map of Atlantic salmon mitochondrial DNA (top line). Variable sites are indicated below line; invariant restriction sited are indicated above line. Locations of sites produced by BstEII and DraI are taken from the map of Bermingham et al. (1988). Various coding regions are shown below the site map, as is a scale measured in kilobase-pairs (bottom line). Symbols denoting restriction enzymes are: BamHI, B; BglII, L; BstEII, T; DraI, D; EcoRI, E; HindIII, H; PstI, P; SacI, S; SacII, C; SmaI, M; XbaI, X.


L B M C H X T C D S H P E X H P P X B D X E D E
L
D
T
U3
12S
16S
U1
U2
C1
C2
A6
C3
U4
U5
U6
C6
0
2
4
6
8
10
12
14
16

recognition sequence) using equation 16 from Nei and Li (1979; the 'site method'). This equation assumes a value for α of 2.0 (α is the ratio of the square of the mean rate of nucleotide substitution to its variance along the mtDNA molecule). Smaller values of α are sometimes adopted for mtDNA, however when genotypes are similar the effect is insignificant (see Carr et al. 1987). The overall estimates of percent sequence divergence among genotypes were calculated from the two separate estimates by weighting according to the number of nucleotides surveyed (Table 12). Pairwise sequence divergence estimates among the four genotypes range from 0.2 - 1.0 percent.

Presence or absence of the variable sites (generated by Bgl II, BstE II, Dra I, Hinc II and Pvu II) in the four mitochondrial genotypes observed are shown at the bottom of Table 11. Fig. 14 shows an unrooted network, generated using the parsimony method, which links the genotypes. The genotypes fall into two clusters due to the perfect association among alternate fragment patterns generated by the enzymes Bgl II, BstE II and Dra I; all genotypes displayed either the 'A' or 'B' pattern for all three enzymes.

As shown in Table 11, two mtDNA genotypes were observed among the sample of anadromous Atlantic salmon while four genotypes were recorded among the nonanadromous salmon. Genotype 1 was the most prevalent mtDNA type and occurred in

Table 11: Distribution of mtDNA genotypes among anadromous and nonanadromous salmon. Letters in each composite genotype represent the fragment patterns generated by individual restriction endonucleases that produce variable patterns. Enzymes are numbered as shown in Table 10. The nucleon diversity index (h) is also given for the two forms. The lower section of the table indicates presence (+) or absence (-) of the variable restriction sites in each of the four mitochondrial genotypes observed.

Genotype	Endonuclease	Anadromous	Nonanadromous	
	1 2 3 4 5			
1	A A A A A	28	23	
2	B B B A A	9	1	$x^2 = 16.7$
3	A A A B A	0	6	$p<0.001$
4	B B B A B	0	4	
	h	0.37	0.52	

	Genotype			
	1	2	3	4
Bgl II	-	+	-	+
BstE II	-	+	-	+
Dra I	-	+	-	+
Hinc II	-	-	+	-
Pvu II	+	+	+	-

76 and 67 percent of anadromous and nonanadromous salmon respectively. Genotype 2 was more prevalent among anadromous salmon (24 vs 3 percent), while genotypes 3 and 4 were observed only among nonanadromous salmon. Genotype frequencies differed significantly between the two forms (x^2=16.67, $p<0.001$).

The nucleon diversity index (h, Nei and Tajima 1983) of the two salmon types is shown in Table 11. Lineage diversity appears to be greater among the nonanadromous sample.

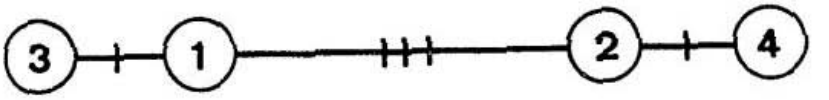
Evaluation of Mitochondrial DNA Techniques

As described previously, different techniques were employed to prepare salmon mtDNA and to visualize restriction fragments after electrophoresis. The degree of nuclear DNA contamination was quite variable among the mtDNA samples prepared using the alkaline extraction method (Palva and Palva 1985). In restriction digests wherein very small mtDNA fragments are not produced, some nuclear DNA contamination does not seriously hamper visualization of fragments using either end labelling or ethidium bromide staining and UV-illumination. Mitochondrial DNA bands in this case are easily seen against the background signal created by nuclear DNA. Similarly, when digested DNA fragments are transferred to nylon membranes and probed with labelled mtDNA, background problems are not encountered.

Table 12: Percent sequence divergence estimates for mtDNA genotypes observed among Gambo salmon (above diagonal). The number of restriction sites observed in each genotype is shown on the diagonal, while entries below diagonal indicate the number of sites shared by genotype pairs.

	1	2	3	4
1	53	0.4	0.2	0.8
2	53	56	0.6	0.2
3	53	53	54	1.0
4	52	55	52	56

Fig. 14: Unrooted network linking Atlantic salmon mitochondrial genotypes using the parsimony method.


3
1
2
4

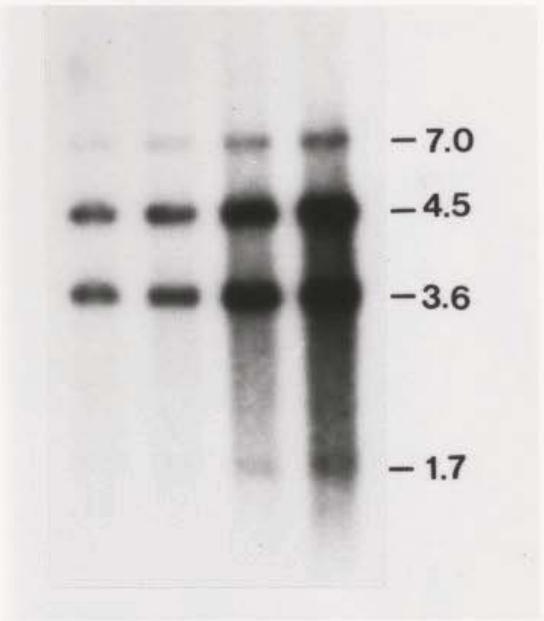
When small mtDNA fragments (e.g. 1 kb or less) are to be visualized, the purity of the mtDNA preparation is more important. Often small fragments are difficult or impossible to observe over background smears when mtDNA is prepared using the alkaline extraction method. In contrast to the alkaline extraction method, mtDNA banded on cesium chloride density gradients was consistently of high purity; the lack of contaminating nuclear DNA eliminates background problems especially when end labelling is employed. This method is generally superior to techniques that do not rely upon density gradient centrifugation when small restriction fragments are to be visualized or when limited amounts of material are available.

Direct visualization of mtDNA restriction fragments in agarose gels using ethidium bromide staining is generally satisfactory when relatively large amounts of mtDNA are available and when small fragments are not scored. When mtDNA is prepared from very small amounts of tissue or when many restriction digests are to be carried out on each sample, the more laborious end labelling procedure becomes necessary. The latter method is extremely sensitive; less than 10 ng of mtDNA are required per digest and small restriction fragments are routinely visualized.

Southern blotting was used for a few samples which were seriously contaminated with nuclear DNA. This technique is considerably more sensitive than ethidium bromide staining

although the disadvantages of increased labour, time and expense are considerable. In most cases mtDNA of high purity prepared from mature capelin oocytes was used as probe. In general this heterologous probe was satisfactory, however visualization of very small fragments is sometimes difficult, probably due to poor binding of small fragments to membranes. In general though, for restriction enzymes with hexanucleotide recognition sequences, capelin mtDNA was a suitable probe (Fig. 15).

Fig. 15: Autoradiogram of Southern blot of Atlantic salmon mtDNA digested with the restriction endonuclease HpaI and probed with ^{32}P-labelled capelin mtDNA. Fragment sizes are indicated on the right; note variable band intensity within samples. See text for blotting and probing conditions.


7.0
4.5
3.6
1.7

DISCUSSION

The central objective of this study was to determine whether sympatric anadromous and nonanadromous Atlantic salmon in the Gambo River system represent separate reproductive units, or whether interbreeding occurs. This information is required for optimal management of salmon in this system and in many others in Newfoundland. For example, assuming that the two forms do not interbreed (and breed true) and that the management objective was simply to generate maximal economic return, then the nonanadromous salmon might not be considered an important component of the salmon resource. Furthermore, since both forms make use of resources within the system that the more economically valuable anadromous salmon could presumably use exclusively, nonanadromous salmon in the system might actually reduce the production of anadromous salmon. In this situation conservation of the nonanadromous fish would not be afforded high priority. On the other hand, if the two forms do interbreed or do not breed true, then at least some of the anadromous salmon are progeny of nonanadromous fish. In this case the latter could be considered as an important component of the resource.

A more enlightened management strategy would consider more than immediate economic concerns and would aim to conserve all genetic variation present in the system (Gilpin and Soulé 1986; Meffe 1987). In this case both anadromous and

nonanadromous components of the salmon resource would be considered worthy of conservation to ensure long term population viability.

Growth and Maturation

At swim-up the mean length of nonanadromous salmon was significantly greater than that of the anadromous salmon. This suggests that important genetically determined differences in life history traits may be present among the two forms of salmon in the Gambo system, contrary to the results of Gross (1987), who observed little difference in life history traits among anadromous and nonanadromous forms of several salmonid species. More detailed life history studies are required to determine whether this is the case.

The force(s) selecting for larger size at swim-up among the nonanadromous salmon are unknown. The two forms appear to occupy different habitats during incubation and early post-emergent stages: under natural conditions anadromous salmon in Triton Brook spawn some distance upstream from the outlet into Gambo Pond South while the nonanadromous salmon spawn at the outlet of Gambo Pond North. Anadromous salmon fry therefore emerge into riverine habitat while nonanadromous fry experience lacustrine habitat upon emergence. Environmental conditions which favour larger young, including predation risk to small size classes and competition for scarce resources (Stearns 1976), may be more important in the pond than in the

river. Adult nonanadromous salmon, present in the ponds but absent in Triton Brook, may apply significant predation pressure on the small size classes. Leggett and Power (1969) reported that forage fish, including salmon, make up the bulk of the diet of adult nonanadromous salmon in Gambo Pond during June and July. Other potential predators including brook charr, Arctic charr and American eels may also consume significant numbers of small nonanadromous salmon in the pond. However, the relative importance of predation as a selective pressure for size at swim-up in the pond and river habitats is not known. Anadromous and nonanadromous fry may move freely between the two habitat types, thereby nullifying potential differential predation pressure. Use of lake habitat by juvenile anadromous salmon is common in Newfoundland (Pepper 1976; Chadwick and Green 1985; Hutchings 1986).

From 1 April 1986 (time of swim-up) until 5 January 1987 (time of grading) consistent differences in growth performance were not observed, although growth was greater among anadromous salmon between 2 April and 2 August. Instantaneous growth rates reveal a period of very rapid growth in both groups during August. Neither group consistently grew more rapidly than the other, hence by grading time mean fork lengths were not significantly different.

Between 5 January and 9 April 1987, growth rates, as reflected by mean fork length and instantaneous growth rates were not significantly different among the two groups of large

size class salmon. After this period instantaneous growth rates were higher among the anadromous salmon, leading to significantly greater mean fork lengths by 5 June. Faster growth during the spring is consistent with the view that the anadromous group underwent parr-smolt transformation to a greater extent than the nonanadromous group. Rapid growth during the spring is an obvious physiological characteristic of smolting salmon (Hoar 1976; Folmar and Dickhoff 1980; Dickhoff and Sullivan 1987; McCormick and Saunders 1987) and has been correlated with neuroendocrine processes. The involvement of thyroid hormones in the parr-smolt transformation was first suggested by Hoar (1939b) who observed enlargement of thyroid follicular cells in smolting Atlantic salmon. More recent work has confirmed Hoar's observations, and elevated levels of thyroid hormones have been measured in smolting Pacific and Atlantic salmon (see Dickhoff and Sullivan 1987 for review). Thyroid hormones promote development in vertebrates and have been shown to increase growth rate in juvenile coho salmon (Dickhoff and Sullivan 1987) and other species. Growth hormone has also been implicated in smolting. Komourdjian et al. (1976a) reported histological evidence for pituitary somatotroph activation in smolting Atlantic salmon, and also observed that salinity tolerance and growth in seawater were higher among juveniles injected with porcine growth hormone relative to saline injected controls (Komourdjian et al. 1976b). Evidence

suggests that reciprocal stimulation of thyroid and growth hormones occurs in smolting salmon (see Folmar and Dickhoff 1980 for references.) While hormone levels were not determined in this study, differences in growth rates observed among cultured anadromous and nonanadromous salmon are probably correlated with differences in seasonal profiles of these hormones. Since culture conditions experienced by the two groups were identical, the different growth rates among large grade fish probably reflect genetic differences.

The distribution of length frequencies of cultured Atlantic salmon appeared unimodal throughout the rearing period. This contrasts with observations made by Saunders et al. (1982) and Thorpe and coworkers, who, in a series of papers, reported bimodal length frequency distributions among sibling populations of Atlantic salmon parr that became apparent by autumn of the first year of life. Parr in the larger modal group were shown to represent salmon destined to smoltify as 1-year-olds while those in the lower modal group were destined to smoltify at two years of age or older (Thorpe 1977). Several factors probably explain differences in growth patterns observed by Thorpe and those reported in this study. Firstly, while Thorpe (1977) does not provide explicit information regarding rearing temperature, heated water was evidently not used in his experiments to accelerate development. Elevated water temperature was used in this study during egg incubation and for several months post-hatch,

providing conditions under which growth was accelerated. Elevated temperature resulted in earlier hatching and commencement of exogenous feeding and enabled the salmon to attain a larger size, on average, by midsummer. Thorpe et al. (1980) have established that during the first summer cultured underyearling salmon parr adopt one of two developmental pathways, i.e. maintenance of high levels of feeding and growth resulting in smolting the following spring, or alternatively reduction of food intake and growth rates resulting in delayed smolting. Segments of the Gambo salmon groups did not appear to reduce growth rate, hence bimodal length frequencies distributions did not develop. Secondly, the cultured Gambo salmon did not represent sibling groups. Thorpe et al. (1980) observed that the timing of the developmental switch, and the size of fish in which the switch occurs, varied among sibling populations of salmon. This variation might be expected to obscure bimodality in length frequency distributions among populations derived from multiple parents. The latter probably does not explain the lack of bimodality in length frequency distributions however, since Bailey et al. (1980) did observe bimodal distributions among non-sib groups of Atlantic salmon.

Different growth rates observed among the large size class anadromous and nonanadromous salmon from April until June might also be related to the high incidence of sexual maturation among the male nonanadromous post-smolts. Ninety-

two percent (12/13) of such males cultured in freshwater after the smolting period and 50 percent (3/6) of these males cultured in seawater matured the following autumn. Maturation was not observed among male post-smolts in the anadromous group, nor among females of either group. This difference between the forms probably reflects genetic differences in response to the same environmental conditions, and suggests that smolting did not occur among nonanadromous males. In general, Atlantic salmon do not migrate to sea as smolts in the spring and mature the following autumn; migrants usually spend at least 12 months at sea before returning to freshwater. Seaward migrants maturing six months after leaving the river would obviously not be in a suitable environment for spawning and the result would be wasted reproductive effort. Smolting followed by sexual maturation in the same year are thus mutually incompatible processes (see Thorpe 1987). There are exceptions to this generalization. Hutchings (1986) observed that some male Atlantic salmon left Wings Brook during the spring smolt migration and returned the following autumn in the sexually mature state. Power (1969) also noted that some salmon in rivers in northern Québec returned to freshwater in the autumn following emmigration.

Condition Factor and Water Content

In several salmonines decreased condition factor is typically associated with smolting (Hoar 1939a, 1976; Fessler

and Wagner 1969; Farmer et al. 1978) and is probably a result of increased growth in length coupled with reduced lipid content (Lovern 1934; Komourdjian et al. 1976a; Farmer et al. 1978; Sheridan et al. 1983). Water content usually varies inversely with lipid content in fishes (Phillips 1969), and elevated water content is usually observed in smolting salmon exhibiting decreasing lipid levels. These changes are usually considered a result of metabolic activation and reorganization associated with expression of adaptations appropriate for life in the marine environment.

In contrast to the typical decrease, anadromous and nonanadromous salmon exhibited increasing condition factor and decreasing water content during the study period. This is almost certainly a function of the culture conditions employed, as the salmon were fed to satiation throughout the experiment. Although total lipid content was not measured, it is very likely that levels increased during the winter and spring. Johnston and Saunders (1981) did not observe decreasing condition factor or lipid content in smolting, cultured Atlantic salmon. Energy requirements for growth, maintenance and smolting processes were evidently met by exogenous food intake in both studies, in contrast with results of other studies reporting reduction in energy stores (including depot lipid, protein and carbohydrate) during smoltification (e.g. Farmer et al. 1978). Such studies suggest that smolting salmon often do not meet these energy

requirements from food intake. Sheridan et al. (1985) have shown that lipid catabolism increases while fatty acid synthesis decreases in several tissues in smolting coho salmon. Disagreement among studies is probably due to culture conditions and/or stock differences.

In mid July 1988, mean moisture content was significantly greater in nonanadromous salmon, although condition factors were virtually identical. The inverse relationship usually observed between lipid content and water content in fishes strongly suggests that fat levels were higher in the anadromous group at this time. Since a high proportion of nonanadromous males matured the following autumn it is possible that lipid reserves in these fish were being channelled into gonad production by this time. This is supported by the more advanced testicular development, evident at the gross level, observed among nonanadromous salmon at this date. Seasonal dynamics of moisture content and condition factor do not reveal differences in smolting physiology among the two groups, however the sharp decline in moisture observed in anadromous salmon between mid June and mid July, and not observed among nonanadromous salmon, is suggestive of genetic differentiation between the two forms, perhaps related to maturation.

Silvering, ATPase and Plasma Na^+ and Cl^-

Clear differences were evident in seasonal patterns of

body silvering of the two forms. While salmon in both groups became more silvery between April and June, the trend was more pronounced among anadromous fish. The proportion of fish in the latter group classified as highly silvered was approximately double that in the nonanadromous group on the sampling dates in June. Differences observed in the proportions of fish classified as unsilvered, partially silvered and highly silvered in May and June probably reflect genetic differences. The silvery coloration characteristic of smolts is due to deposition of purine crystals in the skin, especially beneath the scales and in the dermal layer adjacent to the body musculature (Johnston and Eales 1967). These crystals (guanine and hypoxanthine chiefly) are laid down above, and effectively mask, other pigments residing in the skin. Silvering is the most obvious morphological change that occurs during the parr-smolt transformation and is usually associated with seaward migration. The adaptive value of such coloration to a pelagic marine fish is obvious, however it is less apparent for salmon that remain in freshwater throughout the life cycle. Silvering has been reported in several other populations of nonanadromous salmon (Dahl 1928; Wilder 1947; Barbour et al. 1979; Havey and Warner 1970; Birt and Green 1986). Most nonanadromous populations (including that of Gambo) occupy drainages containing substantial areas of lake habitat which the salmon use extensively (Havey and Warner 1970; Couturier et al. 1986; Hutchings 1986). Such fish

usually undergo a pelagic phase in these lakes during all or some of their lives so silvery coloration is presumably adaptive in this situation as it is in the sea. Silvering therefore, should not automatically be assumed to indicate impending seaward migration in juvenile salmon.

Nonanadromous salmon populations are widely believed to have been derived from anadromous ancestors which dispersed from southern glacial refugia since the end of the Wisconsinin glacial period (Power 1958; Berg 1985). These are therefore young populations (at most 10,000 years old) and might not be expected to have differentiated greatly from their anadromous progenitors in such a short time. Despite their recent development, important genetic differentiation has been noted among nonanadromous forms including early maturation (Sutterlin and MacLean 1984) and suppression of some components of the parr-smolt transformation (Birt and Green 1986). One might expect that nonanadromous salmon would have dispensed with components of the smolt transformation associated with a switch to hypoosmoregulation (such as decreased kidney glomerular filtration rate, increased drinking rate and changes in the gill epithelium associated with salt secretion), yet maintained those alterations adaptive to a pelagic phase (such as increased buoyancy and integumentary silvering). In this regard it is of interest that seasonal levels of Na^+-K^+ ATPase activity were quite different among the two groups. Anadromous salmon exhibited

increasing enzyme activity levels during the winter and spring with a peak in activity occurring at the time of seaward migration. The nonanadromous group also exhibited increasing activity during the winter and spring, however peak levels in May and June were far below those recorded among the anadromous salmon. Consistent with this observation is the finding that mean gill chloride cell size and abundance were greater among the anadromous salmon. Chloride cells excrete excess salts that accumulate while fishes reside in seawater (Kamiya 1972; Foskett and Scheffey 1982) and become more numerous in euryhaline fishes when transferred from freshwater to seawater (Jozuka 1966; Karnaky et al. 1976; Burton and Idler 1984). Absence of a springtime elevation in ATPase activity has also noted in a different population of nonanadromous salmon from Newfoundland (Birt and Green 1986).

The idea that nonanadromous salmon have selectively dispensed with some developmental alterations associated with smolting is tenable if the smolt transformation is viewed as a series of more or less independent changes rather than a single process. As noted above the parr-smolt transformation is a complex series of events which occur over several months in the salmon's life, and which bring about important changes in metabolism, behavior patterns and hydromineral regulation. Much remains to be learned about the hormonal regulation of smoltification although it is clear that hypothalamic, pituitary, thyroid, interrenal and other endocrine tissues are

involved (see Hansen et al. 1989). There is no reason to believe that all these changes evolved at the same time; McCormick and Saunders (1987) show that important differences in smolting physiology are seen among different species of salmonine fishes. Development of salinity tolerance preceeds development of smolt coloration and behavior in steelhead trout (Conte and Wagner 1965; Wagner 1974) and Atlantic salmon (Farmer et al. 1978). Silvering in migratory brook charr develops without concomitant development of hypoosmoregulatory capability (McCormick et al. 1985). This illustrates that physiological changes related to osmoregulation are independent of changes in skin pigmentation and migratory behavior although in a broad sense all these features are considered part of the parr-smolt transformation.

The extent to which other components of the parr-smolt transformation have been lost from the developmental program of the Gambo nonanadromous salmon is not known. Increased buoyancy and reduced willingness to swim against a current are two behavioral alterations that have been noted in the smolting process (Pinder and Eales 1969; Thorpe and Morgan 1978). Further research will be required to determine if these and other changes which promote downstream displacement of smolts and hypoosmoregulatory mechanisms have been maintained in nonanadromous salmon in Gambo River and other populations.

The absence of substantial induction of gill Na^+-K^+ ATPase

activity and increased chloride cell size and number did not appear to compromise the salinity tolerance of cultured nonanadromous salmon. This group survived 96 hours exposure to full strength seawater just as well as the anadromous group. Salmon appeared healthy and active at the end of the exposure period, suggesting that hypoosmoregulatory ability was well developed in both groups. However, despite the high degree of salinity tolerance observed in the two forms, the clearly different seasonal profiles of gill ATPase activity among salmon cultured under identical conditions support the view that genetic differences exist.

Several reports have documented reduction in plasma Na^+ and/or Cl^- concentration in smolting salmon prior to seaward movement (Koch et al. 1959; Houston 1960; Virtanen 1987). Primmett et al. (1988) measured transepithelial Na^+ fluxes in smolting Atlantic salmon. They observed decreasing levels of net Na^+ accumulation from February until April followed by net loss of Na^+ between April and early May and then re-establishment of equilibrium by mid May. This pattern of Na^+ loss (and elevated body water content and urine production [Eddy and Talbot 1985]) in smolts was attributed to a break down in physiological mechanisms of freshwater-related hydromineral regulation. Thorpe (1977) views elevated gill Na^+-K^+ ATPase activity in smolts as a response by poorly adapted fish to elevated body water content and reduced salt levels and as an attempt to restore proper internal

hydromineral balance. According to this hypothesis downstream movement reflects an inability to maintain position within the flow due to osmotic stress rather than directed migration.

This view of the parr-smolt transformation and downstream movement is very different from the traditional one maintaining that alterations associated with smolting occur as preparation for subsequent seaward migration (Hoar 1976; McCormick and Saunders 1987). According to the latter view, salmonine fishes smoltify 'in anticipation' of relocation to the marine environment wherein newly expressed adaptations enhance fitness. On the other hand, Thorpe and his coworkers view seaward migration as the involuntary removal of smolts from the river due to a seasonal breakdown in hyperosmoregulatory capacity leading to reduced swimming ability. According to this hypothesis, smolts do not actively move to sea but rather are washed out by the current. Once in the sea, migrants acclimate to the marine environment before hyperosmoregulatory adaptations become re-established.

Seasonal patterns in plasma Na^+ and Cl^- concentration were not different among the two groups. Both anadromous and nonanadromous salmon experienced more or less constant Na^+ levels during the sampling period except for the mid June sample wherein a sharp reduction was observed. A rather constant decrease in plasma Cl^- concentration was observed from 1 March until the end of the sampling period. Mean plasma Na^+ concentration fluctuated widely from mid May until

mid June suggesting that hydromineral imbalance occurred in both groups, although body water content did not increase in either group during this time. Gill Na^+-K^+ ATPase activity did not appear to respond to correct this situation. Enzyme activity began to increase prior to the period of unstable plasma ion levels, suggesting that Na^+-K^+ ATPase activity was not stimulated to restore normal internal salt levels. In addition, mean plasma Na^+ levels were virtually identical among groups on most sampling dates despite very different Na^+-K^+ ATPase profiles. If the role of ATPase induction in smolting salmon is to restore plasma ion levels then similar seasonal activity levels would be expected among anadromous and nonanadromous groups in light of similar plasma profiles.

Elevated gill Na^+-K^+ ATPase activity in smolting salmon is probably an adaptation related to hypoosmoregulation which develops while the fish is still residing in freshwater. Smolts retained in freshwater after the normal migration time lose most enzyme activity over a period of several months (Johnston and Saunders 1981; Johnston 1983) while smolts transferred to seawater maintain or further increase levels of enzyme activity (Johnston and Saunders 1981; Langdon and Thorpe 1984). Whether elevated ATPase activity in freshwater smolts is actually functional in vivo is unknown. If so, this may contribute to hydromineral imbalance by causing active secretion of salts in an enviromment where salt conservation is necessary. Ultrastructural studies of chloride cells

suggest this may not be the case, since certain alterations of the gill epithelium that are probably associated with salt secretion are not well developed until after exposure to seawater. These alterations include formation of apical crypts and excretory vesicles; exposure to seawater also appears to bring about direct contact of more chloride cells with the external medium. While in freshwater many chloride cells appear to be shielded from the external medium by cytoplasmic projections from associated cells (Lubin et al. 1989).

In summary, progeny of anadromous and nonanadromous salmon from the Gambo River system displayed clear differences in development patterns when cultured under identical conditions. Nonanadromous salmon were larger than anadromous salmon at the time of swim-up. Although growth rates were similar for most of the first year of development, the large size class anadromous salmon grew more rapidly for approximately two months during the spring (April-June). Seasonal profiles of gill Na^+-K^+ ATPase activity were also different. Anadromous salmon exhibited a pattern typical for smolting salmon: enzyme activity was low during early winter but gradually increased until June. July values were much lower than those in May and June, indicating desmoltification. Although gill ATPase activity also increased during the winter and spring in nonanadromous salmon, peak activities were much lower than in anadromous fish. Salinity tolerance was high in

both groups: mortality was very low in April and June after exposure to seawater (32 ppt) for 96 hours. Anadromous salmon exhibited more and larger chloride cells in the gill epithelium at the time of smolting. Similarly, body silvering was more evident among the anadromous group. No differences were noted in seasonal moisture content or in levels of plasma Na^+ or Cl^- concentration. Post-smolt maturation rates were different: 92 percent of nonanadromous males cultured in freshwater during the summer following the smolting period matured while 50 percent of those cultured in seawater matured over the same period. Maturation was not observed among male anadromous post-smolts nor among females of either group. Because these groups were cultured under identical conditions, these developmental differences probably reflect genetic differences and suggest that gene flow between the two forms is restricted to a significant extent under natural conditions.

Mitochondrial DNA

As previously described, mtDNA is generally a more sensitive indicator of population structuring than is protein electrophoresis. Work has been done at the protein level with the aim of delineating population structure of Atlantic salmon over most of its range. The serum protein transferrin was among the first polymorphic proteins identified in Atlantic salmon and some effort has been expended in analysis of the

distribution patterns of the four alleles that have been discovered (Möller 1970; Payne et al. 1971; Payne 1974; Verspoor 1986). Many salmon populations have been genetically characterized more thoroughly. Allele frequencies have been estimated at more than 60 loci in some populations, however the overall level of variation appears to be low. More than 95 percent of electrophoretically detectable variation is accounted for by only four loci (for reviews see Ryman 1983; Ståhl 1983, 1987; Davidson et al. 1989a). Protein electrophoresis has not generally allowed researchers to examine salmon populations with the degree of sensitivity required for differentiation of specific stocks. Most loci are invariant or are variable only in isolated populations. Protein electrophoresis has permitted delineation of three major population clusters. The most important discontinuity is observed between North American and European populations, while a lesser discontinuity separates Baltic populations from those occupying European rivers draining into the Atlantic (Ståhl 1987).

Examination of more variable regions of the genome of Atlantic salmon is necessary. Whether variation in the mitochondrial genome is sufficient to provide stock-specific markers is not known, however it is probable that the degree of discrimination sensitivity will exceed that provided by protein electrophoresis. This has been illustrated in several studies by Avise and coworkers in which mtDNA analysis was

conducted in conjunction with conventional protein electrophoresis. Population structuring in pocket gophers (Geomys pinetis) in the south eastern U.S. is not apparent from morphological criteria, and electrophoretic examination of protein variation revealed only one fixed difference among 25 loci. In contrast, five of six restriction endonucleases were able to differentiate between the two races present (Avise et al. 1979). Similarly, two subspecies of bluegill sunfish (Lepomis macrochirus) in the southeast U.S. exhibit fixed differences at two nuclear loci. The same subspecies differ by 20 restriction site changes as determined using restriction site maps generated by 12 restriction endonucleases (Avise et al. 1984). Ward et al. (1989) examined genetic variation in walleye (Stizostedion vitreum) populations from the Great Lakes and northern Manitoba. These authors showed that at least 10% of the total variation at five polymorphic nuclear loci was attributable to interpopulation variation while approximately 30-50% of the total mtDNA variation was explained by interpopulation variation. These examples and others on record demonstrate the greater sensitivity of mtDNA analysis compared to conventional protein electrophoresis for detecting population structure.

Very little information concerning mtDNA variation in Atlantic salmon has been published. A limited survey of two allopatric populations in Newfoundland (one anadromous, one

nonanadromous) revealed almost no variation among a small number of salmon (Birt et al. 1986). In a somewhat more extensive survey Palva (1986) observed clear differences in mtDNA of anadromous salmon from the Neva River and nonanadromous salmon from the River Pielisjoki, Finland. Hovey et al. (1989) screened 40 salmon from the River Itchen in southern England for approximately 150 restriction sites generated by eight enzymes; three enzymes produced variable fragment patterns and a total of six mtDNA genotypes were observed.

Evidence for large scale structuring of mtDNA variation in Atlantic salmon was reported by Bermingham et al. (1988). These authors characterized mtDNA variation in approximately 20 salmon derived from hatcheries in New England and New Brunswick, and approximately 25 salmon from various European hatcheries. North American and European mtDNA genotypes were found to differ by at least seven restriction sites, and apparently fixed differences were identified by five restriction enzymes (BglI, BstEII, ClaI, DraI, AvaII). To test the generality of this observation a further sample of 68 salmon, all of which had been tagged as smolts (thus whose natal river was known) and were subsequently caught in the sea off western Greenland were analysed with this set of enzymes. In 67 of these salmon the continent of origin as determined using mtDNA analysis was in agreement with the known origin of the fish.

On the basis of these findings it appeared that the contribution of European and North American stocks to the Greenland fishery could be estimated accurately. However, results of the present study do not support the observations of Bermingham et al. (1988). Both "European" and "North American" genotypes (as identified using the enzymes BglII, BstEII and DraI) were observed among the Gambo salmon and have been observed in salmon from other populations in Newfoundland (Davidson et al. unpublished). The differences between European and North American populations reported by Bermingham et al. (1988) are not fixed, and are probably an artifact of the limited sample of hatchery fish used in their initial characterization of mtDNA variation. Use of this data to estimate the contributions to the high seas fishery of European and North American salmon stocks probably underestimates the proportion of North American salmon since some fish displaying the "European" genotype would in reality represent salmon of North American origin. Accurate characterization of mtDNA variation in salmon from a larger fraction of the species' range on both sides of the Atlantic is clearly required before stock composition can be determined in waters off west Greenland and elsewhere.

The mitochondrial genome size in Atlantic salmon estimated in this study (16.7 kb) agrees closely with sizes reported in other studies (Birt et al. 1986; Palva 1986; Gyllensten and Wilson 1987; Bermingham et al., 1988; Hovey et

al., 1989). With regard to other salmonids, Berg and Ferris (1984) reported a mitochondrial genome size of 16.67 kb in chinook salmon (Oncorhynchus tshawytscha), rainbow trout (O. mykiss), brown trout (Salmo trutta) and brook charr. Grewe and Hebert (1988) observed a genome size of 16.8 kb in lake charr (Salvelinus namaycush). Genome size for several nonsalmonid fishes are quite similar to these values. Bluegill sunfish was found to have a slightly smaller mitochondrial genome (16.2 kb; Avise et al. 1984) as was the domestic goldfish (Carassius auratus; Beckwitt and Aoyagi 1987). In contrast, genomes from fishes of the family Scorpaenidae appear to be somewhat larger and more variable in size, ranging from 17.2-19.5 kb in Sebastes melanostomus and Scorpaena guttata respectively (Beckwitt and Petruska 1985).

The level of mtDNA clonal diversity (expressed as nucleon diversity, h) was greater in the nonanadromous than anadromous Gambo salmon (Table 11). Gyllensten and Wilson (1987) found that clonal diversity within European populations of brown trout was generally from 0.6-1.0, somewhat greater than in Atlantic salmon. This observation is consistent with the greater degree of morphological variation in brown trout over its range.

The level of sequence divergence estimated among the four mtDNA genotypes observed in Gambo salmon (0.2-1.0 percent) is similar to that observed among other salmonine species. Wilson et al. (1985) found that sequence divergence among

mtDNA genotypes in several populations of rainbow trout ranged from 0.135-1.50 percent, while that in chinook salmon ranged from 0.13-0.81 percent (Wilson et al. 1987). In lake charr from the Great Lakes, sequence divergence among mtDNA genotypes is similar (0.180-1.177 percent; Grewe and Hebert 1988). Atlantic herring (Clupea harengus) exhibit somewhat greater sequence divergence among mtDNA genotypes (0.19-4.37 percent; Kornfield and Bogdanowicz 1987). In general sequence divergence among rodent mtDNA genotypes appears to be greater than in fishes, with mean values of 1.7 and 4.2 percent for the meadow vole (Microtus pennsylvanicus, Plante et al. 1989) and the rat (Rattus rattus, Brown and Simpson 1981) respectively. If, as Bermingham et al. (1988) suggest, the major discontinuity in the Atlantic salmon mtDNA lineage network (Fig. 14) represents differentiation between European and North American mtDNA genotypes, the time elapsed since they last shared a common ancestor can be estimated. By plotting the estimated mtDNA sequence divergence of species pairs against the time elapsed since each pair shared a common ancestor (as determined using the fossil record), Brown et al. (1979) estimated that the mean rate of sequence divergence among pairs of mtDNA lineages in great apes is approximately two percent per million years. Subsequent sequencing of specific mitochondrial genes from the same group of animals confirmed this rate estimate (Brown et al. 1982). Shields and Wilson (1987) employed this approach with several species of

geese and obtained the same rate of sequence divergence. Assuming that this rate applies to Atlantic salmon it is estimated that the European and North American genotypes diverged approximately 350,000 years ago, during the Yarmouth Interglacial (Cornwall 1970). Estimates of divergence times in mtDNA lineages must be regarded with caution at this time. The rate of nucleotide sequence divergence in mtDNA was determined using a limited group of species, all of which are warm-blooded. The possibility that different nucleotide substitution rates apply to cold-blooded organisms cannot be rejected at this time in light of recent evidence suggesting that rates of amino acid substitution in proteins encoded by the mitochondrial genome are greater in birds and mammals than in fishes (Kocher et al. 1989; Thomas and Beckenbach 1989). Further research in this regard is clearly needed to clarify this matter.

Results of the mtDNA study do not support the hypothesis that anadromous and nonanadromous salmon in the Gambo system constitute a single reproductive unit. These forms should therefore be managed as separate stocks. The various mtDNA genotypes are present in the two groups in significantly different frequencies, demonstrating that successful exchange of breeding females is rare (assuming no selective advantage among genotypes). That the two forms are reproductively isolated is further supported by the apparent genetic basis for developmental and physiological differences observed among

the cultured salmon.

Isolating Mechanisms

The genetic differences observed among the two forms is evidence that interbreeding is infrequent. Assuming the absence of differential selection on mtDNA genotypes, heterogeneity in genotype frequencies can be maintained only under very low levels of gene flow (Slatkin 1987). What factors operate to maintain reproductive isolation in the Gambo system? There is evidence that temporal mechanisms operate in this system. Adult broodfish from Triton Brook and from Gambo Pond North were captured at nearly the same time of year, yet the anadromous females ovulated at least two weeks earlier than nonanadromous females. The time intervals during which the two forms spawn naturally in the Gambo system are not known, however a two week difference in spawning times could potentially reduce gene flow between the populations. There are published accounts of sympatric populations of other salmonids maintaining reproductive isolation by spawning at different times of the year. The Arctic charr of Windermere are subdivided into at least two isolated populations, one of which spawns during the autumn (November), the other during the spring (February-March). The contention that these forms are reproductively isolated is supported by the observation of highly significant differences in the frequencies of two serum esterase alleles (Child 1984). Leider et al. (1984) reported

that sympatric populations of summer and winter-run steelhead trout in the Kalama River, a tributary of the Columbia River, are largely reproductively isolated, despite use of common spawning areas, by spawning at different times of year. Based upon observations made on tagged steelhead trout over a three year period, these authors found that the mean spawning date among wild summer-run fish was 15 February while that for wild winter-run fish was 14 April. Although reproductive isolation is not complete, they concluded that racial identity is maintained by this mechanism. A relatively minor difference in mean spawning dates, such as that suggested among the Gambo salmon, probably could not bring about the degree of reproductive isolation required to prevent homogenization of mtDNA genotype frequencies, however, this coupled with other mechanisms (such as preference for different spawning sites) could result in almost complete isolation of the two forms.

The two forms appear to use different areas of the system as spawning sites. As previously described, the anadromous stock in question spawns in Triton Brook, the major tributary of Gambo Pond South, while the nonanadromous salmon spawn at the outlet of Gambo Pond North. Anadromous salmon are known to spawn at other sites within the system (Porter et al 1974). Approximately 25 - 40 percent of the anadromous salmon in the system spawn in Mint Brook. While the majority of the remaining anadromous fish spawn in Triton Brook, redds have been observed in gravel beds at the narrows during years of

low water discharge. Spawning of anadromous salmon is also suspected in Parson's Brook despite several potentially serious barriers to upstream migration (Porter et al. 1974). Spawning activity has also been reported in the two-kilometer stretch of river between the outlet of Gambo Pond North and Freshwater Bay.

From what is known about the distribution of spawning activity throughout the system, spawning habitat utilized by anadromous salmon appears typical of that normally associated with the species i.e. gravel beds in relatively fast flowing water (Jones 1959). Nonanadromous salmon on the other hand, appear to deposit their eggs in areas of reduced flow, specifically in North Pond near the outlet. Thus, different habitat selection at spawning time might effectively reduce gene flow between the populations to the degree that heterogeneity of mtDNA genotype frequencies (and nuclear gene frequencies) is maintained. Couturier et al. (1986) have determined that nonanadromous salmon in North Arm River, Newfoundland, use areas of lake outlet as spawning sites. These authors employed ultrasonic tracking to follow the movements of mature nonanadromous salmon and found that the fish congregated around the outlet of a lake for some time during the spawning season. Subsequent observations by divers revealed that ova had been scattered among rocks and crevices on the lake bottom up to a distance of approximately 20 meters from the outlet. Anadromous salmon in this system constructed

redds in the main stem of the river some distance from the spawning site occupied by nonanadromous salmon. The two forms appear to use different spawning habitats, and the nonanadromous salmon are egg scatterers rather that nest builders. How widespread these differences are in Newfoundland and elsewhere is not known although typical redd building by nonanadromous salmon in riverine habitat has been reported elsewhere (Havey and Warner 1970).

Spatial and temporal reproductive isolation of sympatric populations have also been reported in Arctic charr (Skúlason et al. 1989). Four morphotypes of this species are present in an Icelandic lake (Thingvallavatn). The large benthivorous form is restricted to spawning sites where cold groundwater enters the system, while the other forms are not confined to such sites. Selection of alternate spawning sites based upon groundwater input has also been observed in populations of kokanee (Oncorhynchus nerka) in Lake Kronotskiy, Kamchatka (Kurenkov 1977).

Assortative mating is another potential isolating mechanism. Evidence for assortative mating in Atlantic salmon has not been observed, however the phenomenon does occur in sockeye salmon (Oncorhynchus nerka). Foote and Larkin (1988) found that male anadromous and nonanadromous (kokanee) sockeye prefer mates of similar size. Streamside observations of spawning fish revealed a complete absence of anadromous males courting territorial kokanee females, although kokanee males

courted large anadromous females. Since the anadromous salmon in the Gambo system (and in most other rivers) are much larger than the sympatric nonanadromous salmon assortative mating based on size could effectively limit gene flow between forms. In mating experiments, Hutchings and Myers (1985) found that anadromous and nonanadromous Atlantic salmon will interbreed and these authors concluded that behavioral isolating mechanisms do not prevent such matings in nature. However adult salmon were 'force paired' in this study; given the opportunity, spawning adults might have chosen mates of similar size. Hutchings and Myers (1985) showed that prezygotic isolation of the two forms based upon nonrecognition of courtship signals does not prevent interbreeding among forms.

Prezygotic isolating mechanisms associated with mate selection that operate among anadromous and nonanadromous sockeye salmon (and potentially in Atlantic salmon) appear to be based upon male choice. Foote (1988) established that mate selectivity in male kokanee increased with male size. Since female attractiveness appears to increase with female size, intrasexual competition among males effectively reduces the availability of large females to small males. This is reinforced by female preference for large males (Foote and Larkin 1988). The end result is assortative mating based upon size, and since kokanee are typically smaller than anadromous sockeye, the latter will tend not to interbreed with the

kokanee. The same mechanism may be important in isolating anadromous and nonanadromous Atlantic salmon in the Gambo system.

While nonanadromous spawners appear to be confined to the area near the outlet of Gambo Pond North, anadromous spawners are not restricted to Triton Brook; a small number of ripe anadromous fish were captured at the lake outlet. Several hypotheses can explain this observation. The anadromous specimens may have been simply passing through the area en route to some other spawning site within the system. On the other hand, both forms may spawn at the same locality in the pond and interbreed freely. If this is the case then the apparently separate gene pools identified by the mtDNA analysis and culture experiment are not based primarily upon the anadromous/nonanadromous dichotomy, but rather on reproductive isolation resulting from fidelity to alternate spawning sites. The culture experiment lends some support to this hypothesis; while mean springtime gill Na^+-K^+ ATPase activity levels were much higher among offspring of anadromous parents, some nonanadromous individuals did have activity levels indicative of functional anadromous smolts. Similarly the high salinity tolerance among both groups as demonstrated by the salinity challenge tests demonstrates that, despite other indications of loss of smolting capacity, progeny of nonanadromous salmon are capable of acclimation to sea water. This hypothesis implies that at least two salmon stocks occupy

the Gambo system and that alternate life histories (anadromy v.s. residency) are employed by individuals of the largely nonanadromous stock (Gross 1984).

Alternatively, assortative mating could occur at the pond outlet as it does in sympatric sockeye and kokanee in Pierre Creek (Foote and Larkin 1988), implying that the anadromous/nonanadromous dichotomy is the basis for the separate gene pools. Under this hypothesis anadromous salmon spawning at the pond outlet may or may not belong to the Triton Brook spawning population. Conceivably, several distinct salmon stocks are present in the Gambo system, however, whether homing behavior among spawners is sufficiently accurate to maintain reproductive isolation is not known.

One argument supporting the hypothesis of reproductive isolation among the two forms is that of 'hybrid' disadvantage. Conditions are obviously very different in the marine and freshwater environments so it is reasonable to assume that selection pressures encountered by the two life history types are dissimilar. Different selective forces may have molded different adaptive features in each form. Interbreeding might then result in 'hybrid' offspring that are less well adapted to either environment than the parental types. This phenomenon of outbreeding depression has not been reported in natural populations of salmonids although it has been observed in other animals (Clarke et al. 1968; Alstad and

Edmunds 1987). Many examples of heritable, stock-specific differences are known in Atlantic salmon (e.g. Gjedrum and Aulstad 1974; Glebe et al. 1979; Riddell et al. 1981; Saunders 1981; Ståhl 1987) and outbreeding could conceivably alter these traits and lead to reduced fitness of 'hybrid' offspring. Further research is required in this regard.

Despite differences in mtDNA and developmental patterns, some gene flow between forms may occur. A potential avenue for gene flow is the movement of male spawners (especially precocious males) among spawning sites. Precocious maturation is widespread among male Atlantic salmon in Newfoundland (Dalley et al. 1983) and the phenomenon is probably common in the Gambo system. Introgression due to the movement of males among spawning populations would not be detected by mtDNA analysis since mtDNA is inherited through the female parent. Despite this possibility, clear differences evident in development and smolting physiology (processes which are almost certainly governed by nuclear genes) suggest that the two forms differ genetically in important ways. Determination of nuclear gene frequencies using protein electrophoresis or some other molecular technique might indicate whether successful 'straying' by mature males occurs within the system.

The occurrance in sympatry of genetically differentiated populations of various salmonine fishes has been documented previously. The most pronounced tendency in this regard is

seen among the charrs (Salvelinus spp.). Arctic charr have the widest natural distribution of any salmonine fish and exhibit wide phenotypic (and genotypic) variation. Genetically distinct sympatric populations have been reported in lakes throughout northern Europe and Siberia. Behnke (1972) argued that, based upon the degree of morphological differentiation among some sympatric populations, reproductive isolation developed during preglacial times, while in other cases isolation occurred since the most recent glacial period. Among the former group the highly divergent 'tiefseesaibling' from the Bodensee and several other European Alpine lakes are considered by Behnke to be separate species. In some lakes sympatric charr populations use different spawning sites. In a situation analogous to that among salmon in Gambo Pond, one charr population in Lake Taimyr spawns in tributary streams while a second population of smaller fish utilizes lake habitat for spawning. In many lakes containing sympatric S. alpinus populations, a large piscivorous form coexists with one or more smaller (often dwarfed) forms which feed primarily upon benthic or planktonic invertebrates (Nordeng 1983; Skúlason et al. 1989).

The other species of Salvelinus have more restricted ranges than S. alpinus, and fewer lakes containing sympatric genetically distinct populations are known. Sympatric populations of S. malma have been described from Kamchatka in Lakes Azabachye and Dalnye. Similarly, distinct populations

of S. namaycush occur in the Great Lakes basin (Ihssen et al. 1988). Two lakes are known to have contained separate populations of Salvelinus fontinalis. Dublin Pond in New Hampshire was home to the silver charr prior to its extinction due to introgression of introduced exotic stock during the early years of this century. The aurora charr is the second well differentiated form of S. fontinalis and occupies White Pine Lake, Ontario. Both the silver and aurora charrs are thought to represent relicts of lineages that diverged in preglacial times (Behnke 1972).

Genetically distinct sympatric populations are known within the genus Oncorhynchus. The best known examples are seen in sockeye salmon. As described above, distinct populations of anadromous sockeye and nonanadromous kokanee are found in Babine Lake (Skeena drainage) and in lakes in the Fraser and Columbia drainages (Foote et al. 1989). Smith (1969) reported evidence for genetic differentiation between summer and winter races of steelhead trout in the Cipilano and other rivers in British Columbia. Similarly, Leider et al. (1984) concluded that gene flow between summer and winter-running stocks of steelhead was sufficiently low to allow maintenance of separate stocks. Genetic differentiation of sympatric steelhead and rainbow trout as determined by mtDNA analysis supports the contention that inbreeding among the two forms is not extensive (Wilson et al. 1985b).

Within the genus Salmo, the best known example of

reproductive isolation among sympatric populations is probably the brown trout in Lough Melvin, Ireland. Three distinct morphotypes in addition to the 'common brown trout' are present in Lough Melvin: ferox, a large piscivorous form; sonaghen, the black-finned trout; and gillaroo, the red-spotted trout. Ferguson and Mason (1981) observed heterogeneous allele frequencies among morphotypes and heterozygote deficiencies among pooled brown trout samples. These findings constitute strong evidence for reproductive isolation among morphs, although the isolating mechanisms are not known.

Reproductive isolation has also been demonstrated among sympatric brown trout populations in Lake Bunnersjoarna, a small mountain lake in Sweden (Ryman et al. 1979). Two populations within the lake are fixed for alternate alleles at a lactate dehydrogenase locus, and exhibit significant allele frequency differences at other loci. A further example of this phenomenon was described by Numann (in Behnke 1972). Two morphologically dissimilar brown trout types occupy Lake Garda, Italy: a typical lacustrine population of relatively large trout and a small form occupying deep water. The latter is a benthic form in which two annual spawning seasons occur during June and December.

In contrast with the great phenotypic variation observed in S. trutta over its range, Atlantic salmon are morphologically uniform. According to Behnke (1972) 'no other

salmonid species of comparable geographic distribution appears so phenotypically uniform throughout its range' (p. 650). Consistent with this uniformity, direct evidence supporting the existence of genetically distinct sympatric populations of Atlantic salmon has been published only recently. Vuorinen and Berg (1989) determined that separate populations of nonanadromous salmon occupy the River Namsen in Norway. These populations are not prevented from mingling by barriers to upstream migration, yet significant allele frequency differences indicate that gene flow is very low or absent. Similarly, Verspoor and Cole (1989) found significant heterozygote deficiencies relative at two protein-coding loci, and significant nonrandom associations between genotypes at five loci in Atlantic salmon sampled in a lake on the Gander River system, Newfoundland. These observations suggest that samples were drawn from a mixture of two separate gene pools, possibly corresponding to anadromous and nonanadromous populations. Couturier et al. (1986) provided evidence for spatial reproductive isolation among anadromous and nonanadromous Atlantic salmon in North Arm River, Newfoundland. The present study provides an additional example of the coexistence of distinct salmon populations whose integrity is probably maintained by faithful homing to separate spawning sites (philopatry).

Evolution of Nonanadromous Salmon

The evolution of nonanadromous Atlantic salmon populations appears to have occurred during the short time since the withdrawal of Wisconsin glaciers. As discussed by Power (1958), populations in the southern part of the North American range (i.e. Sebago salmon) are situated along the fringe of the maximal extension of the continental ice sheets. As glacial ice advanced southward, rivers containing salmon were disrupted and their fish populations exterminated. When the climate warmed at the close of the glacial period rivers began to flow again as ice sheets retreated northward. The climatic warming was probably not uniform; warm periods were interspersed with relatively cold periods. As salmon straying from southern unglaciated streams populated the new northern rivers they probably experienced periodic episodes of prolonged cold conditions. As described by Power (1958), two characteristics of salmon in the Ungava Bay area (the northern limit of distribution) are the large size and old age of smolts undertaking seaward migration. Cold conditions that probably occurred at the close of the Pleistocene may have had the effect of reducing the migratory tendency to a greater extent than it reduced sexual maturation. A portion of the salmon under such cold conditions (females in particular) might be expected to mature without prior seaward migration. Further glacial withdrawal, and frequently the formation of barriers to upstream migration by the rising of the land,

effectively isolated these new freshwater populations in headwater regions and imposed strong selection for the nonmigratory habit. The postglacial origin of nonanadromous salmon populations in northern Europe is indicated by the recent and rather synchronous formation of barriers to upstream migration present in several rivers (Berg 1985). Furthermore the overall morphological uniformity among anadromous and nonanadromous salmon argues for the recent origin for the latter. This contrasts with several populations of charrs and coregonine fishes that appear to have survived glaciation in various lacustrine refugia, and later came into sympatry with more phenotypically 'normal' populations via various dispersal routes (Behnke 1972).

The nonanadromous salmon in Gambo Pond show that evolution of the freshwater-resident life history does not necessarily require a physical barrier to upstream migration of anadromous salmon. Gambo Pond North lies at an elevation of only four meters, and the approximately two kilometers of river flowing from the pond outlet to Freshwater Bay follows a very shallow incline. The presence of a waterfall or some other barrier to migration in this stretch of river since the Wisconsinin glacial period is most unlikely. Anadromous salmon in the Gander and North Arm Rivers in Newfoundland are not prevented from contact with the nonanadromous salmon living in lakes in these systems. Since the nonanadromous salmon in these rivers (and many others in Newfoundland)

probably evolved in situ from anadromous salmon occupying the same rivers, and independently from nonanadromous salmon in other rivers, it follows that reproductive isolation can come about in the absence of physical barriers.

The common feature of river systems containing nonanadromous salmon appears to be the presence of lakes. Only one exception is known: the River Namsen, Norway, wherein nonanadromous salmon (smablank) complete their entire life cycle under riverine conditions. The exact role played by lakes in the evolution of nonanadromous stocks is not clear. Berg (1985) suggested that these salmon are able to adopt a pelagic lifestyle in lakes analogous to that of anadromous salmon in the sea. Lake use would facilitate the abandonment of anadromy since the basic life history would not be altered. The only difference would be that adjustments in hydromineral regulation would not be necessary. The success of introduced anadromous Pacific salmonines in the Great Lakes basin and the former presence of Atlantic salmon in Lake Ontario (Netboy 1968) clearly demonstrate that lacustrine habitat can substitute for the marine environment. In the course of evolution, normal anadromous smolts may have simply remained in lakes during the period of seaward movement. Since the physiological capacity of smolts to acclimate to seawater is temporary, significant delay of downstream movement would result in onset of the desmolting process. Re-establishment of adaptations for life in freshwater would then prevent such

fish from successfully leaving the system.

Sympatric, reproductively isolated populations are common in salmonids. The evolutionary divergence of many sympatric conspecific lineages probably occurred prior to the recession of Wisconsinin ice sheets. Among these relatively old lineages initial genetic differentiation likely took place under conditions of allopatry; invasion of lakes by different lineages probably explains their sympatry at present (Behnke 1972). In contrast, other sympatric conspecific lineages appear to have differentiated genetically in situ during the last approximately 10,000 years. This appears to be the case for Atlantic salmon in particular. There is no evidence for the existence of North American refugia wherein nonanadromous salmon could have survived the glacial periods that characterized the Pleistocene. Extant populations within the glaciated areas have therefore originated since the end of the Wisconsin and are derived from anadromous fish which dispersed northward (probably along the coast) after glacial withdrawal. Many nonanadromous populations evolved after the formation of barriers which isolated salmon in headwater regions from those situated further downstream. The genetic divergence that has occurred among the two forms within these rivers has not taken place in strict allopatry since one-way movement of fish from above barriers to downstream areas almost certainly occurs (Vuorinen and Berg 1985) in spite of strong directional selection that must counter downstream movement. In this case

extrinsic barriers do keep the nonanadromous salmon reproductively isolated from other populations. These populations may be subject to population bottlenecks, genetic drift and unique selection pressures, all of which can facilitate the evolution of unique gene pools. These phenomena do not appear to affected the nonanadromous salmon in Gambo since the level of mtDNA lineage diversity is comparable to levels observed in other salmonines (and exceeds that observed in the anadromous sample).

Genetically distinct populations that are not separated by impassable falls, such as those in the Gambo system, may give the impression that genetic divergence among lineages has come about under conditions of sympatry. Given sufficient time and assuming that reproductive isolation among the forms is maintained, they will continue to diverge genetically. Do these populations then represent examples of sympatric speciation? To the extent that the forms occupy the same drainage and are not physically separated, they are sympatric. However, at spawning time, different habitat preferences appear to effectively isolate breeding individuals of the two forms in different parts of the system. They are therefore allopatric at the critical time, i.e. when reproduction occurs, and therefore there is a spatial component in their reproductive isolation. Since the breeding populations are not separated by a great distance this speciation might best be termed 'microgeographic' (Mayr 1976).

As described by Foote and Larkin (1988), the anadromous/nonanadromous dichotomy observed among salmonine fishes is a polymorphism that satisfies several requirements of sympatric speciation models. The behavioral polymorphism observed among the Gambo salmon is to a great extent responsible for a size polymorphism, since anadromous fish can take advantage of greater food resources present in the sea and consequently attain a greater size than fish that remain in freshwater. This behavioral polymorphism is probably genetic in nature as indicated by differences noted in smolting physiology among cultured salmon. Assortative mating based upon size, while unknown in Atlantic salmon, has been documented in sympatric sockeye and kokanee, and represents a potentially effective means of restricting gene flow among the forms. Sexual selection is therefore an adequate intrinsic isolating mechanism (West-Eberhard 1983, 1986). While the studies of Foote and Larkin (1988), Foote (1988) and Foote et al. (1989) indicate clearly that sexual selection can prevent genetic homogenization among sympatric anadromous sockeye and nonanadromous kokanee, whether sympatric speciation has in fact occurred is unclear since it is uncertain whether mechanisms of mate selection (and hence lineage divergence) evolved in sympatry or in allopatry. The distribution of electrophoretically detectable genetic protein variation indicates that, in general, sockeye and kokanee within a river are genetically more similar to each other than to populations

from other rivers, indicating that the divergence of anadromous/ nonanadromous lineages occurred within each river. While the divergence leading to the sympatric lineages of O. nerka may have occurred in different areas within each drainage (i.e. allopatric speciation), the possibility of sympatric differentiation cannot be rejected.

Further research will be necessary to determine whether genetic differentiation is occurring in sympatry in Atlantic salmon. Specifically, studies are required to investigate in greater detail the use of separate spawning sites by the two forms in rivers where physical barriers to migration do not prevent interbreeding. As well, studies designed to determine the degree to which sexual selection/assortative mating occurs and is responsible for maintaining barriers to gene flow are in order. Finally, since the mtDNA analysis cannot rule out gene flow resulting from movement of males between breeding sites, further biochemical genetic studies concentrating on nuclear-encoded genes are needed. Sympatric populations of anadromous and nonanadromous Atlantic salmon that are not isolated by physical barriers are a good model for investigating sympatric speciation because of the short time span since lineage divergence. This eliminates the possibility that the nonanadromous populations are derived from occupants of local glacial refugia and reduces the probability that the alternate forms arose by multiple colonizations of northern rivers. By ruling out these two

possibilities, one may assert with some confidence that divergence of the two forms of Atlantic salmon occupying a given river occurred in situ and perhaps in sympatry.

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